(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/21172 A1

(51) International Patent Classification[7]: A61K 31/20, 31/202, A61P 35/00

(21) International Application Number: PCT/AU00/01137

(22) International Filing Date:
18 September 2000 (18.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2914 17 September 1999 (17.09.1999) AU

(71) Applicant *(for all designated States except US)*: **WOMEN'S AND CHILDREN'S HOSPITAL ADELAIDE** [AU/AU]; 72 King William Road, North Adelaide, S.A. 5006 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FERRANTE, Antonio** [AU/AU]; 72 King William Road, North Adelaide, S.A. 5006 (AU). **EASTON, Christopher, J.** [AU/AU]; 72 King William Road, North Adelaide, S.A. 5006 (AU). **HII, Charles, S., T.** [AU/AU]; 72 King William Road, North Adelaide, S.A. 5006 (AU).

(74) Agent: **MADDERNS**; 1/64 Hindmarsh Square, Adelaide, S.A. 5000 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

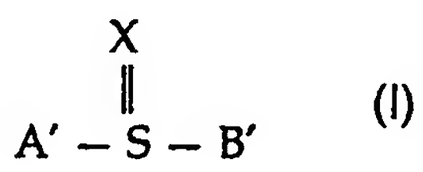
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/21172 A1

(54) Title: ANTI-CANCER NITRO- AND THIA-FATTY ACIDS



(I)

(57) Abstract: The present invention relates to pharmaceutical compositions comprising, as an anti-cancer agent: (a) one or more compounds having the formula NO_2-A-B, wherein A is a saturated or unsaturated hydrocarbon chain of 14-26 double bonds, and B is $(CH_2)_m(COOH)_n$ in which n is an integer from 0 to 2 and m is an integer from 0 to 2; or a derivative thereof in which the hydrocarbon chain has one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy; (b) one or more compounds selected from polyunsaturated fatty acids (PUFA's) having a 16 to 26 carbon atom chain and 3 to 6 double bonds, and wherein the PUFA is covalently coupled at the carboxylic acid group to an amino acid selected from glycine and aspartic acid; (c) one or more compounds selected from unsaturated fatty acids having an 18 to 25 carbon atom chain and 1 to 6 double bonds and wherein the fatty acid has one or two β-oxa, γ-oxa, β-thia, γ-thia substitutions; or (d) one or more compounds having formula (I) wherein A' is a saturated or unsaturated hydrocarbon chain of 9-26 carbon atoms, X is oxygen or is absent and B' is $(CH_2)_j(COOH)_k$ in which j is an integer from 1 to 3 and k is 0 or 1; or a derivative thereof in which the hydrocarbon chain has one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy; and a pharmaceutically acceptable carrier or diluent.

FIELD OF THE INVENTION

The present invention relates to compounds which include a carbon chain of 14 to 26 carbon atoms and a nitro or sulphur group. In a particular embodiment the invention relates to nitro analogues of polyunsaturated fatty acids. The present invention further relates to the use of these compounds in methods of treatment.

BACKGROUND OF THE INVENTION

Fatty acids are one of the most extensively studied classes of compounds due to their important role in biological systems [1,2]. Hundreds of different fatty acids exist in nature. They consist of saturated, monounsaturated and polyunsaturated fatty acids, having chain lengths from 4 to 22 carbon atoms. Polyunsaturated fatty acids (PUFAs) contain 16 to 22 carbon atoms with two or more methylene-interrupted double bonds. The PUFA, arachidonic acid, contains 20 carbons and four methylene-interrupted *cis*-double bonds commencing six carbons from the terminal methyl group, which therefore leads to an abbreviated nomenclature of 20:4 (n-6).

PUFAs can be divided into four families, based on the parent fatty acids from which they are derived: linoleic acid (18:2 n-6), α-linolenic acid (18:3 n-3), oleic acid (18:1 n-9) and palmitoleic acid (16:1 n-7). The n-6 and n-3 PUFAs cannot be synthesised by mammals and are known as essential fatty acids (EFAs). They are required by mammalian bodies indirectly through desaturation or elongation of linoleic and α-linolenic acids, which must be supplied in the diet.

EFAs have a variety of biological activities. For instance, it has been suggested that they are important modulators of neoplastic development because they are capable of decreasing the size and number of tumours as well as the lagtime of tumour appearance.[3] Intake of n-3 PUFAs has been found to be associated with a reduced incidence of coronary arterial diseases, and various mechanisms by which n-3 PUFAs act have been proposed.[4,5] Some n-3 and n-6 PUFAs also possess antimalarial [6] or anti-inflammatory properties.[7] Furthermore, one of the EFAs'

most important biological roles is to supply precursors for the production of bioactive fatty acid metabolites that can modulate many immune functions.[8]

Arachidonic acid (AA) is the most extensively studied of the EFAs and it is a principal precursor for many important biological mediators. There are two pathways for arachidonic acid metabolism (1) the cycloxygenase pathway which leads to the formation of prostaglandins and thromboxanes, and (2) the lipoxygenase pathway which is responsible for the generation of leukotrienes and lipoxins. These metabolites, collectively called eicosanoids, have been implicated in the pathology of a variety of diseases such as asthma [9] and other inflammatory disorders. [10,11]

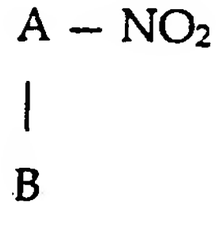
Although EFAs play important roles in the biological process of the mammalian body, they are not widely used as therapeutics due to their limited availability *in vivo*. They are readily degradable by β-oxidation, which is the major oxidative pathway in fatty acid metabolism. The net process of β-oxidation is characterised by the degradation of the fatty acid carbon chain by two carbon atoms with the concomitant production of equimolar amounts of acetyl-coenzyme A.

To overcome the problem of β-oxidation, some work has been done to design and synthesise modified PUFAs, such as the β-oxa and β-thia PUFAs[12,13]. These compounds were shown to have enhanced resistance to β-oxidation while still retaining certain biological activities of the native PUFAs.

The present invention relates to another group of modified PUFAs, the nitro analogues of PUFAs. The rationale was that the nitro group is chemically similar to COOH group with regard to size, charge and shape. In addition, the nitro compounds are a group of relatively stable compounds and are resistant to β-oxidation by preventing CoA thioester production, which is the first step in β-oxidation of fatty acids. This also means that the nitro compounds will not be incorporated into lipids and will more likely be present in a free form.

SUMMARY OF THE PRESENT INVENTION

A first group of compounds of the present invention have the general formula I:-



in which A is a saturated or unsaturated hydrocarbon chain of 14 to 26 carbon atoms; and B is $(CH_2)_n(COOH)_m$ in which n is 0 to 2 and m is 0 to 2;

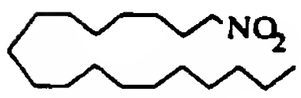
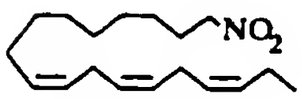
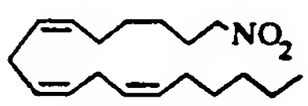
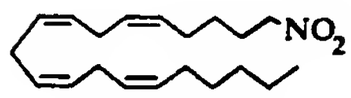
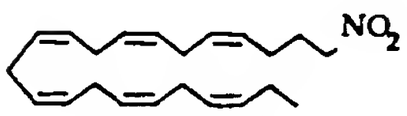
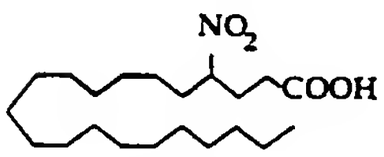
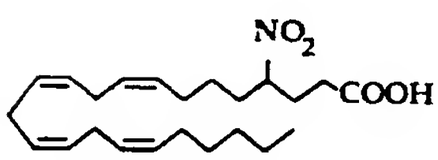
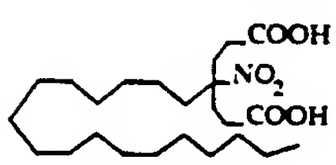
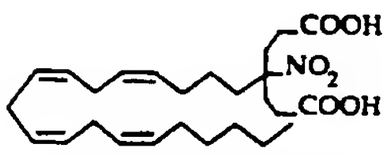
and the derivatives thereof having a further one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

In a preferred embodiment of the present invention, A is a hydrocarbon chain of 18 to 22 carbon atoms which is preferably polyunsaturated, and in particular has 3-6 double bonds.

More preferably, the compound has an unsaturated hydrocarbon chain having 18 carbon atoms and three double bonds separated by methylene groups, with the first double bond relative to the omega carbon atom being between the 3rd and 4th or 6th and 7th carbon atoms.

In a further preferred embodiment, the compound is selected from the group consisting of those set out in Table 1.

Table 1. Structure and nomenclature of nitro fatty acid analogues

Structure	Systematic Name	WCH	Report	Thesis
 19:0-NO_2	1-Nitrooctadecane	Lx1	4a	55
 19:3 (n-3)-NO_2	(z,z,z)-1-Nitro-9,12,15-octadecatriene	Lx2	4c	60a
 19:3 (n-6)-NO_2	(z,z,z)-1-Nitro-6,9,12-octadecatriene	Lx3	4d	60b
 21:4 (n-6)-NO_2	(all-z)-1-Nitro-5,8,11,14-eicosatetraene	Lx4	4b	60c
 23:6 (n-3)-NO_2	(all-z)-1-Nitro-4,7,10,13,16,19-docosahexaene	Lx5	4e	60
 21:0γ-NO_2	4-Nitrohenicosanoic acid	Lx6	6a	80
 23:4 (n-6)γ-NO_2	(all-Z)-4-Nitrotricosa-8,11,14,17-tetraenoic acid	Lx7	6b	82
 γ,γ(COOH), 19:0-NO_2	3-Heptadecyl-3-nitropentane-1,5-dicarboxylic acid	Lx8	8a	84
 γ,γ(COOH), 21:4 (n-4)-NO_2	3-[(all-Z)-Nonadeca-4,7,10,13- tetraenyl] -3-nitropentane-1,5-dicarboxylic acid	Lx9	8b	86

In yet a further preferred embodiment, the compound is Lx2 or Lx3.

In yet a further preferred embodiment, the compound is Lx7 or Lx9.

The ability of the compounds of the present invention to inhibit lipoxygenase activity suggests their use in the treatment of cancer, eg prostate cancer.

They may also find application in the treatment of cancer eg prostate cancer.

The metabolism of arachidonic acid has been a topic of great interest, particularly in relation to its role in inflammation. A major interest has been the search for selective inhibitors of the various enzymes in the arachidonic acid cascade. This is critical for the development of compounds with therapeutic potential for control of the pathological processes mediated by arachidonic acid metabolites, and is also important in providing useful biochemical tools for mechanistic investigation of the enzymes involved. Considerable effort in this area has been made in association with the cycloxygenase pathway, and a number of nonsteroidal anti-inflammatory drugs (e.g. aspirin and indomethacin) have been found to have inhibitory effects on cycloxygenase.[14] More recently, efforts have been extended to a study of the lipoxygenase (LO) pathway and the search for selective inhibitors of the enzymes involved in the pathway. Another major objective of the present work is to assess the possible activity for enzyme inhibition or other potential physiological activities of the synthetic nitro compounds using enzymological and biological assays.

In a first aspect, the present invention consists in an anti-cancer pharmaceutical composition comprising at least one compound of formula I and a pharmaceutically acceptable carrier or diluent.

In a second aspect, the present invention consists in a method of treating cancer (eg prostate cancer) in a subject, the method comprising administering to the subject a therapeutic amount of a compound of formula I.

In a third aspect, the present invention consists in an anti-cancer pharmaceutical composition comprising at least one compound being a polyunsaturated fatty acid (PUFA) having a 16-26 carbon atom chain and 3-6 double bonds, and wherein the PUFA is covalently coupled at the carboxylic acid group to

an amino acid selected from glycine and aspartic acid. These PUFA analogues are described in International Patent Specification No. PCT/AU95/00717.

In a fourth aspect, the present invention consists in a method of treating cancer in a subject, the method comprising administering to the subject a therapeutic amount of such a PUFA analogue.

In a fifth aspect, the present invention consists in an anti-cancer pharmaceutical composition comprising at least one compound being a PUFA having an 18-25 carbon atom chain and 1-6 double bonds and wherein the PUFA has one or two substitutions selected from the group consisting of β-oxa, γ-oxa, β-thia and γ-thia. These compounds are described in International Patent Specification No. PCT/AU95/00677.

In a sixth aspect, the present invention consists in a method of treating cancer in a subject, the method comprising administering to the subject a therapeutic amount of such an oxa- or thia- fatty acid.

In a seventh aspect, the present invention consists in an anti-cancer pharmaceutical composition comprising at least one compound of the formula

$$A - \underset{\displaystyle X}{\overset{\displaystyle \|}{S}} - B$$

wherein A is a saturated or unsaturated hydrocarbon chain of 9-26 carbon atoms, X is oxygen or is absent and B is $(CH_2)_j\,(COOH)_k$ in which j is an integer from 1 to 3 and k is 0 or 1; or a derivative thereof in which the hydrocarbon chain includes one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

In an eighth aspect, the present invention relates to a method of treating cancer in a subject, the method comprising administering to the subject a therapeutic amount of such a thia or sulfinyl fatty acid.

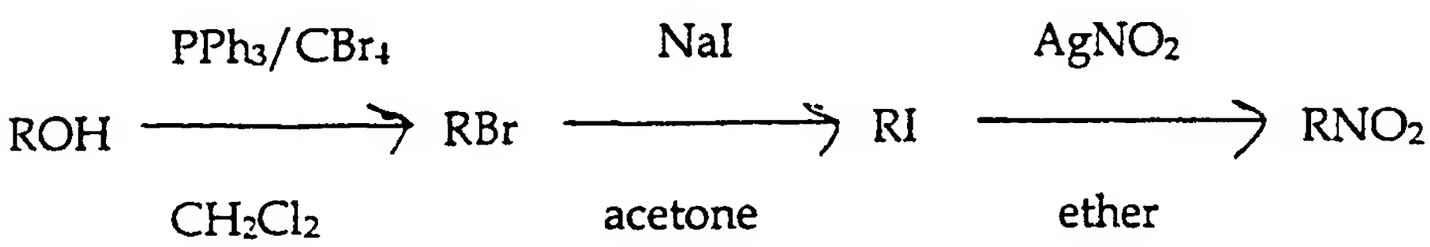
In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples.

A. PREPARATION OF NITRO ANALOGUES OF PUFA

(1) Synthesis of nitroalkanes/nitroalkenes (Lx1 to Lx5)

The first target compounds were a series of nitro compounds with chain lengths of 18 to 22 carbons and 3 to 5 double bonds, being prepared by modification of commercially available polyunsaturated fatty alcohols. Since the unsaturated alcohols are relatively expensive to obtain, stearyl alcohol was used as the starting material for establishing synthetic methods.

The synthesis of nitroalkanes/nitroalkenes [15] Lx1 to Lx5 is summarised in Scheme 1.



(Lx1) 4a: $R=CH_3(CH_2)_{17}$-

(Lx2) 4c: $R=CH_3CH_2(CH=CHCH_2)_3(CH_2)_7$-

(Lx3) 4d: $R=CH_3(CH_2)_4(CH=CHCH_2)_3(CH_2)_4$-

(Lx4) 4b: $R=CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_3$-

(Lx5) 4e: $R=CH_3(CH_2)(CH=CHCH_2)_6(CH_2)_3$-

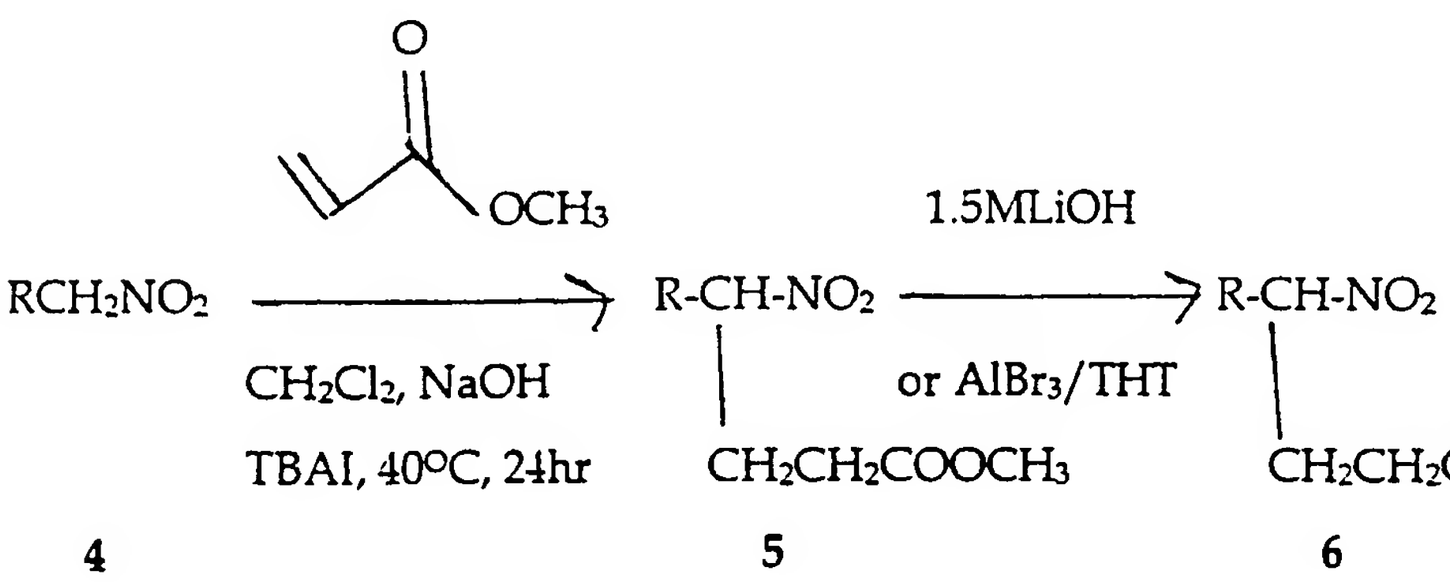
Scheme 1

Stearyl alcohol 1a was converted to stearyl bromide 2a by treatment with triphenyl phosphine (PPh_3) and carbon tetrabromide (CBr_4) in dichloromethane overnight at room temperature. After purification by flash chromatography on silica gel, stearyl bromide 2a was obtained in 96% yield. Treatment at the stearyl bromide with silver nitrate in ether afforded stearyl nitrate 4a in low yield (<10%). Attempts to improve the yield of the nitroalkane 4a from this procedure by extending reaction time and increasing the amount of silver nitrate used were unsuccessful and so conversion of

the bromide to the nitroalkane via the iodide was investigated. Conversion of stearyl bromide 2a to the corresponding iodide 3a was achieved in the yields of >90% as estimated by the ^1H NMR spectrum of crude reaction mixture. Stearyl iodide 3a was converted *in situ* to stearyl nitrate 4a, by treatment with silver nitrate in ether for 3 days at room temperature, and the product, stearyl nitrate 4a, was obtained in 65% yield. Based on this approach, nitroalkenes 4b-4e were synthesised and fully characterised (Scheme 1).

(2) Synthesis of γ-nitroalkanoic and γ-nitroalkenoic acids [6a (Lx6) and 6b (Lx7)]

The synthetic nitroalkane and nitroalkene (Lx1 and Lx4) were further used as starting material for synthesis of γ-nitroalkanoic and γ-nitroalkenoic acids (Lx6 and Lx7). The γ-nitroalkanoic and γ-nitroalkenoic acid esters 5a and 5b were produced by Michael addition of the respective nitroalkane and nitroalkene 4a and 4b to methyl acrylate. The esters were then hydrolysed to give the γ-nitroalkanoic and γ-nitroalkenoic acids 6a and 6b (Scheme 2):



a: $R=CH_3(CH_2)_{16}$-

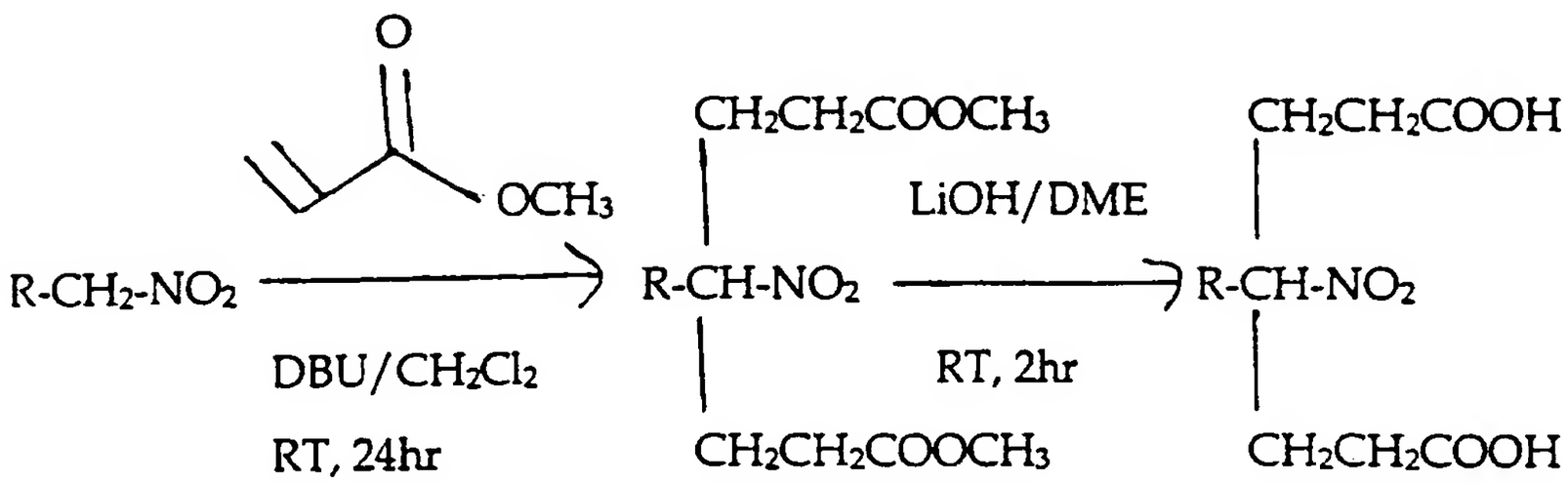
b: $R=CH_3(CH_2)_4(CH=CH-CH_2)_4(CH_2)_2$-

Scheme 2

A published method [16] for the synthesis of short chain γ-nitroalkanoic acid esters was investigated for synthesis of the long chain acid ester 5a. The nitroalkane 4a was treated with methyl acrylate in a two phase system of water and dichloromethane in the presence of sodium hydroxide at room temperature for 24 hours. No reaction occurred under these conditions and a modification was then made where tetrabutylammonium iodide (TBAI), a phase transfer catalyst, was introduced into the reaction to improve the solubility of the base in the organic phase. With this change, a small amount of the expected product was detected by ^1H NMR analysis of the crude reaction residue. The yield of γ-nitroalkanoic acid ester 5a was further improved (reaching 69% yield) by increasing the relative amount to 3:1 (for methyl acrylate : nitroalkane) and by increasing the reaction temperature to 50°C. The γ-nitroalkanoic acid ester 5a was hydrolysed by treatment with either 1.5M lithium hydroxide in dimethoxyethane (DME) or aluminium tribromide in tetrahydrothiophene (THT) at room temperature to afford the γ-nitroalkanoic acid 6a in 98% yield. The unsaturated nitroalkenoic acid 6b was generated in similar yield using the same method, and both 6a and 6b were fully characterised.

(3) Synthesis of α, α-dipropanate nitroalkane and nitroalkene [8a (Lx8) and 8b (Lx9)]

Multiple Michael addition to primary nitroalkanes can lead to the production of multiply substituted nitroalkanes. [17] Based on this, the α, α-dipropanate ester nitroalkane and nitroalkene 7a and 7b were prepared by Michael addition of the nitroalkane and nitroalkene 4a and 4b to methyl acrylate in the presence of 1,8-diazabicyclo [5,4,0] undec-7-ene (DBU) as a strong base. The resulting diesters 7a and 7b were converted to the corresponding dicarboxylic acids 8a and 8b by lithium hydroxide hydrolysis (78-80% yield) (Scheme 3):



4 7 8

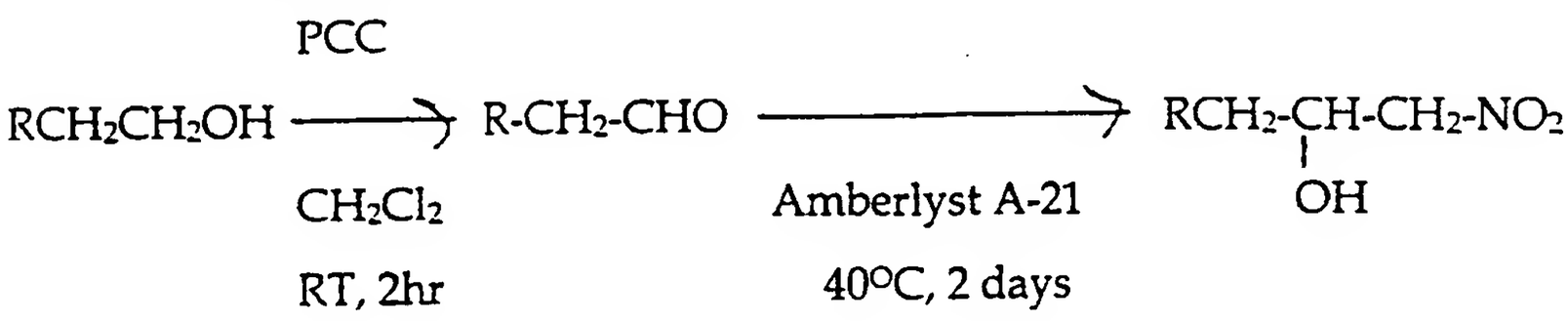
a: $R=CH_3(CH_2)_{16}$-

b: $R=CH_3(CH_2)_4(CH=CH-CH_2)_4(CH_2)_2$

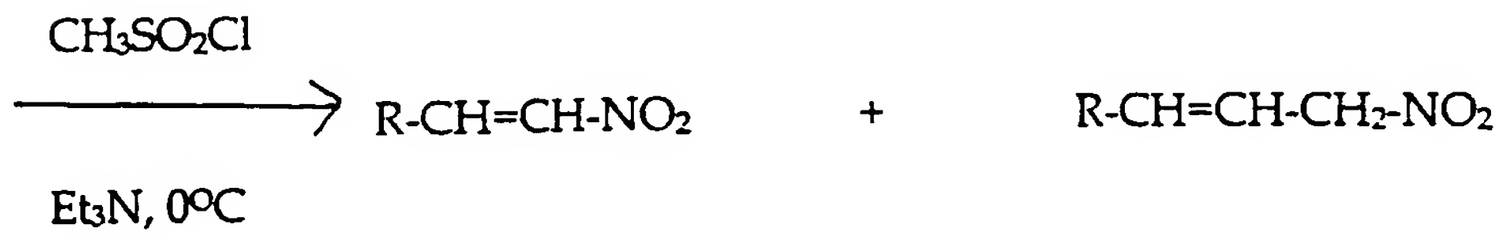
Scheme 3

(4) Synthesis of α, β-unsaturated nitroalkenes (11a and 11b)

The reaction scheme shown below (Scheme 4) was envisaged for generation of α, β-unsaturated nitroalkenes.



1 9 10



11 12

a: $R=CH_3(CH_2)_{15}$-

b: $R=CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)$-

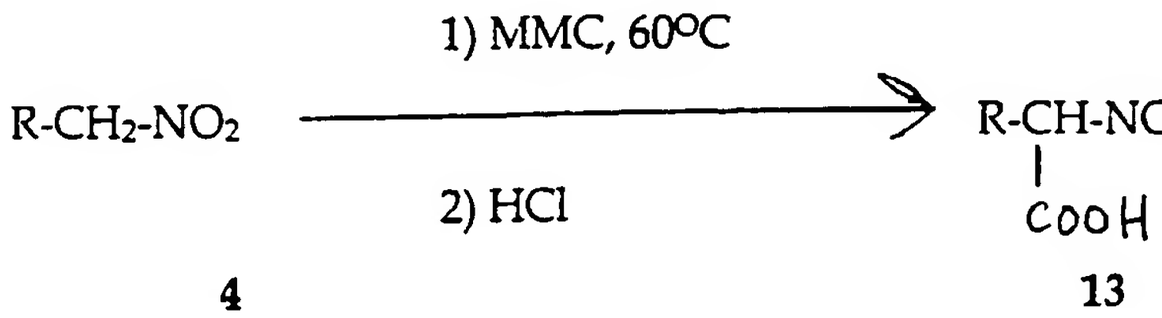
Scheme 4

Fatty alcohol 1a was oxidised by pyridinium chlorochromate (PCC) in dichloromethane at room temperature to yield corresponding aldehyde 9a. [18] β-hydroxy nitroalkane can be efficiently obtained by nitroaldol reaction, [19] and in this case, the aldehyde 9a reacted with nitromethane in ether, with Amberlyst A-21 as a heterogeneous basic catalyst, generating the β-hydroxy nitroalkanes in 89% yield after purification. Dehydration of β-hydroxy nitroalkane 10a [20] was undertaken by mixing with 1 equivalent of methanesulfonyl chloride (CH_3SO_2Cl) and 4 equivalents of triethylamine in dry dichloromethane at 0°C. The ^1H NMR spectrum of the residue indicated that the products were a mixture of conjugated and nonconjugated nitro compounds. In subsequent experiments, this reaction was monitored by TLC from 5 mins to 2.5 hours. The result showed that only the conjugated product 11a could be seen at 5 mins, and after 10 mins of reaction, the nonconjugated product 12a showed up and it became predominant after 2 hrs reaction. Although conjugated 11a and nonconjugated nitro compound 12a were distinguishable by ^1H NMR and ^{13}C NMR, and were separable by TLC, no pure samples of either compound were obtained by flash chromatography due to decomposition. A similar result was obtained for synthesis of conjugated compound 11b.

The variation in the product distribution (11a and 12a) during reaction may be explained on the basis of kinetic versus thermodynamic control. It is possible that the nonconjugated compound 12a is thermodynamically more stable, but the formation of the conjugated product 11a is kinetically favoured over that of the nonconjugated product 12a. However, once the reaction for conjugated compound formation reached a kinetic equilibrium, formation of the nonconjugated compound will become predominant because of its higher thermodynamic stability. However, further work is needed to elucidate this.

(5) Synthesis of α-nitro acids 13a

A reported [21] one-pot method for synthesis of α-nitro acids was investigated which involved the use of magnesium methyl carbonate (MMC) as a carboxylating agent to introduce a carboxyl group at the α-carbon of a primary nitroalkane (Scheme 5):



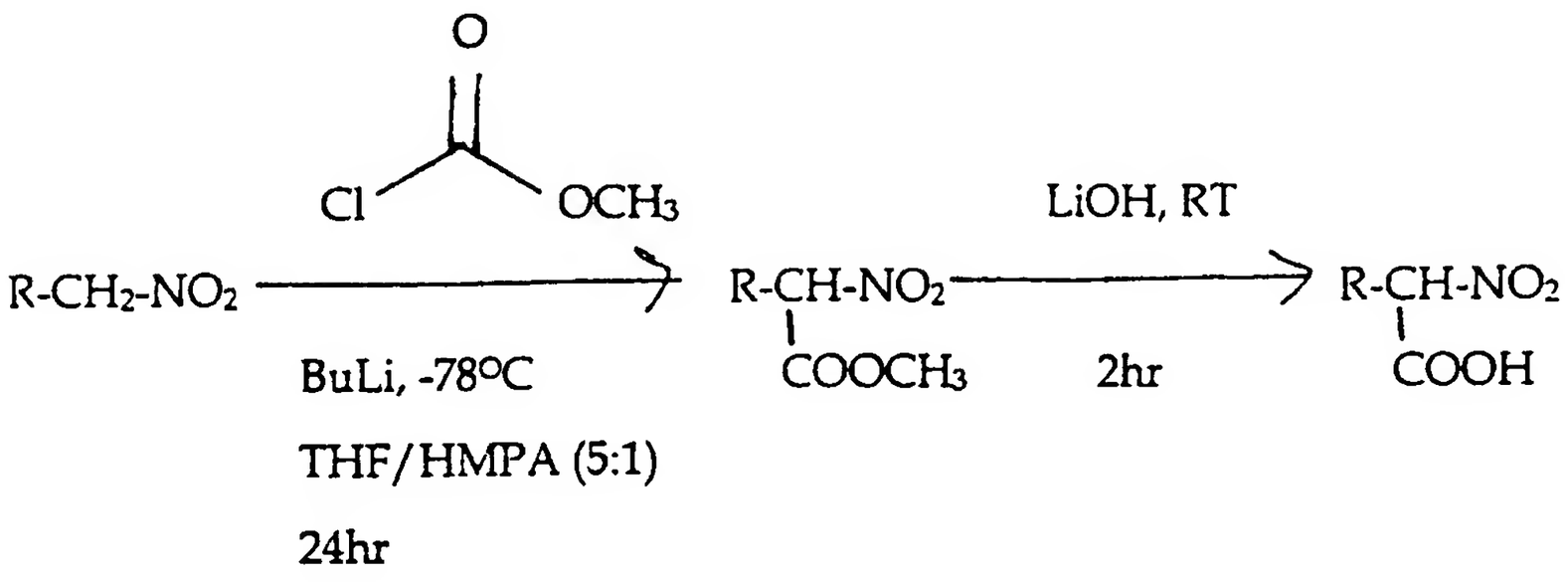
4 **13**

a: $R=CH_3(CH_2)_{16}$-

Scheme 5

When 1-nitropropane was used as the starting material, the ^1H NMR of the residue obtained after workup indicated formation of the corresponding α-nitro carboxylic acid. However, when the long chain nitroalkane 4a was used as the starting material, the expected α-nitro acid product 13a was not detected in the crude reaction mixture. The lack of reaction for stearyl nitrite may be attributed to poor solubility of stearyl nitrite in MMC solution.

Synthesis of the α-nitro acids 14a was subsequently investigated by conversion of the nitroalkane 4a of the corresponding α-nitro acid ester 14a by treatment with methyl chloroformate, followed by hydrolysis (Scheme 6):

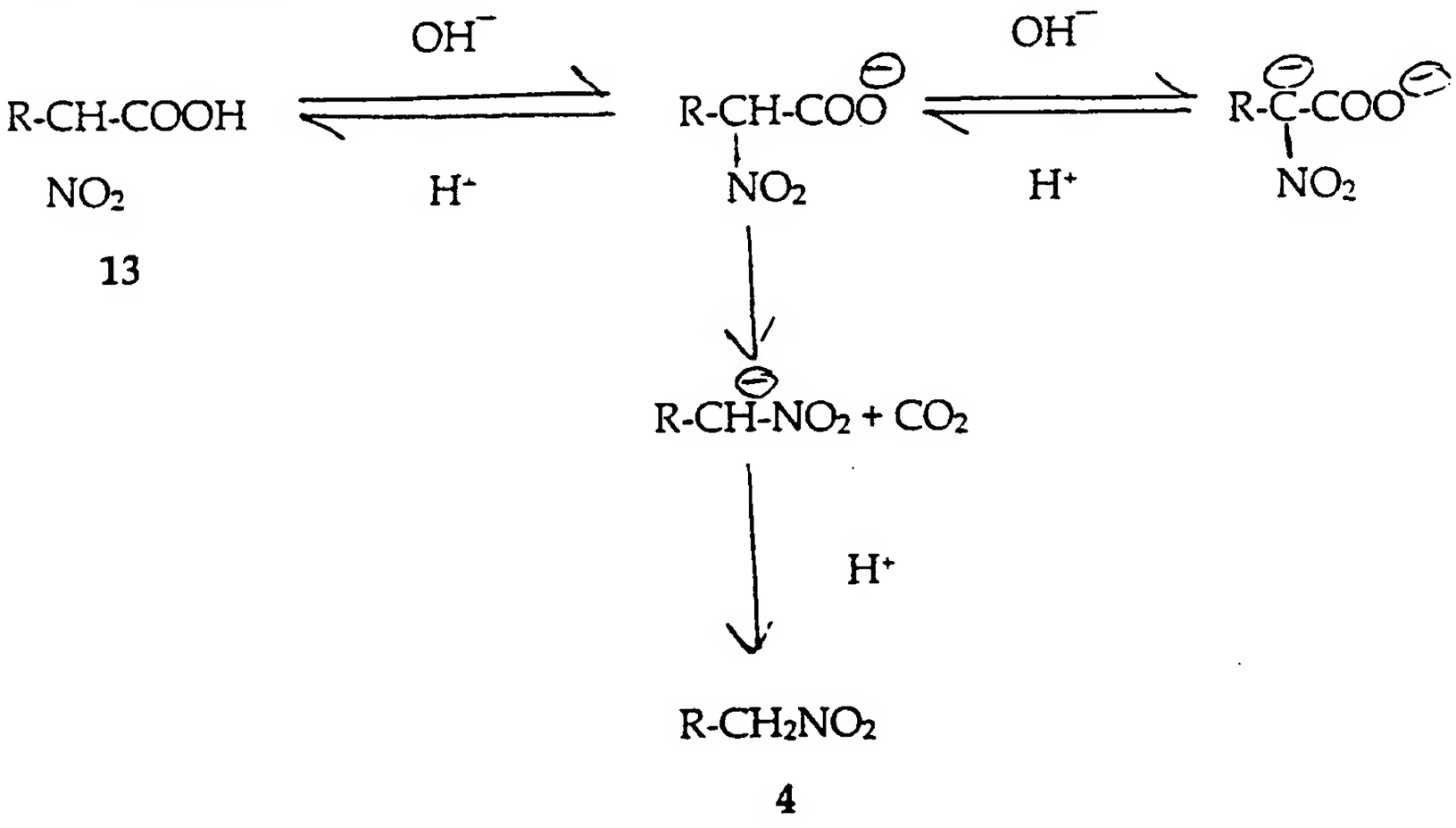


4 **14** **13**

a: $R=CH_3(CH_2)_{16}$-

Scheme 6

Using this scheme, the saturated nitro acid ester 13a was obtained in 25% yield from the corresponding nitroalkane 4a. Treatment of the ester 14a with lithium hydroxide in dimethoxyethane (DME) did not give rise to the desired acid 13a. The nitroalkane 4a, however, was isolated as the sole product of this reaction. This result can be explained as illustrated in Scheme 7.



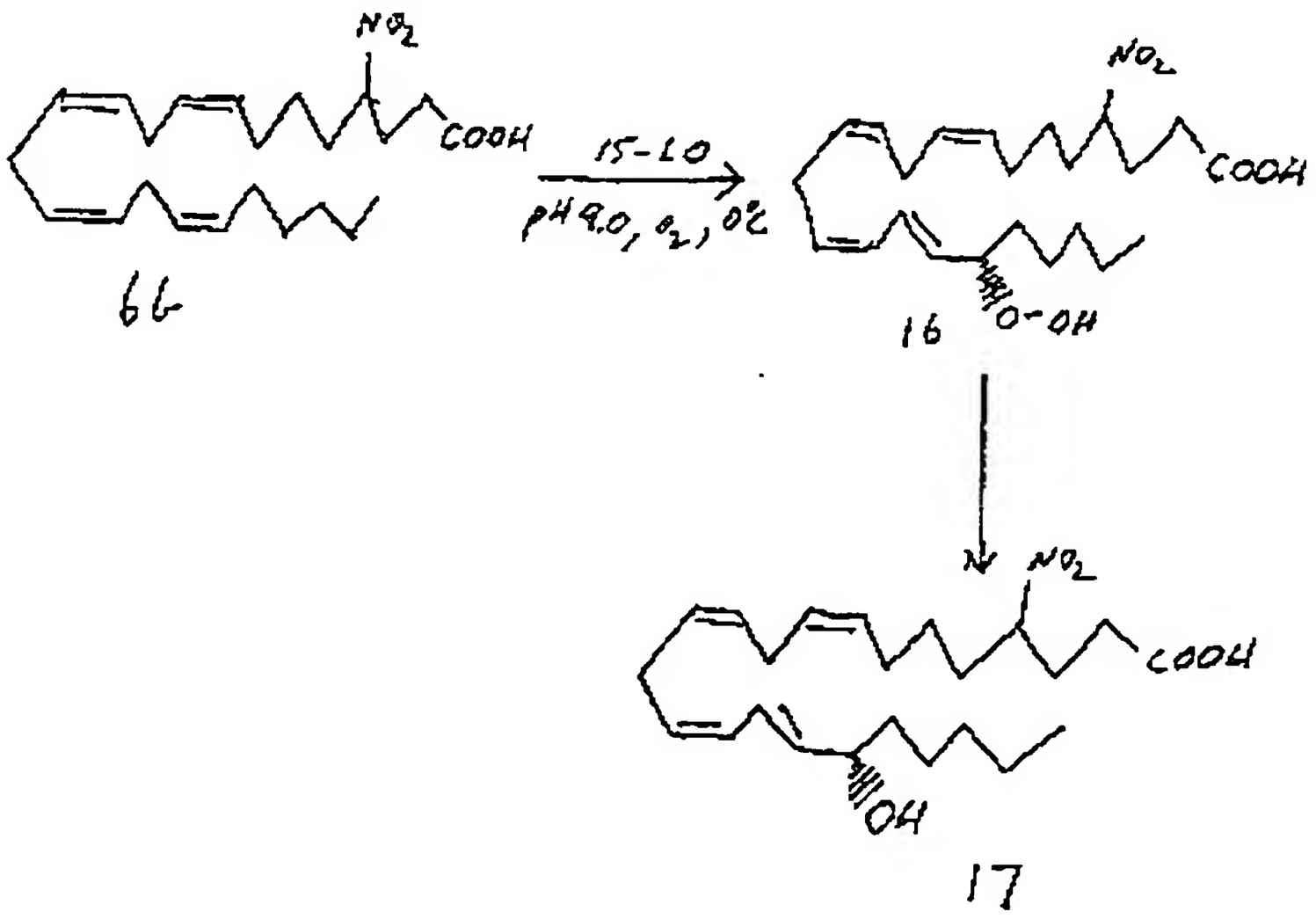
a: $R=CH_3(CH_2)_{16}$-

Scheme 7

It has been reported [22] that free α-nitroacetic acid and its dianion salt are quite stable at room temperature, but that the monoanion salt decarboxylates rapidly at room temperature. The failure in generating the α-nitropropanoic acid is then likely due to decarboxylation of the monoanion in the basic reaction medium.

(6) Synthesis of hydroxy and hydroperoxy derivatives of compound 6b

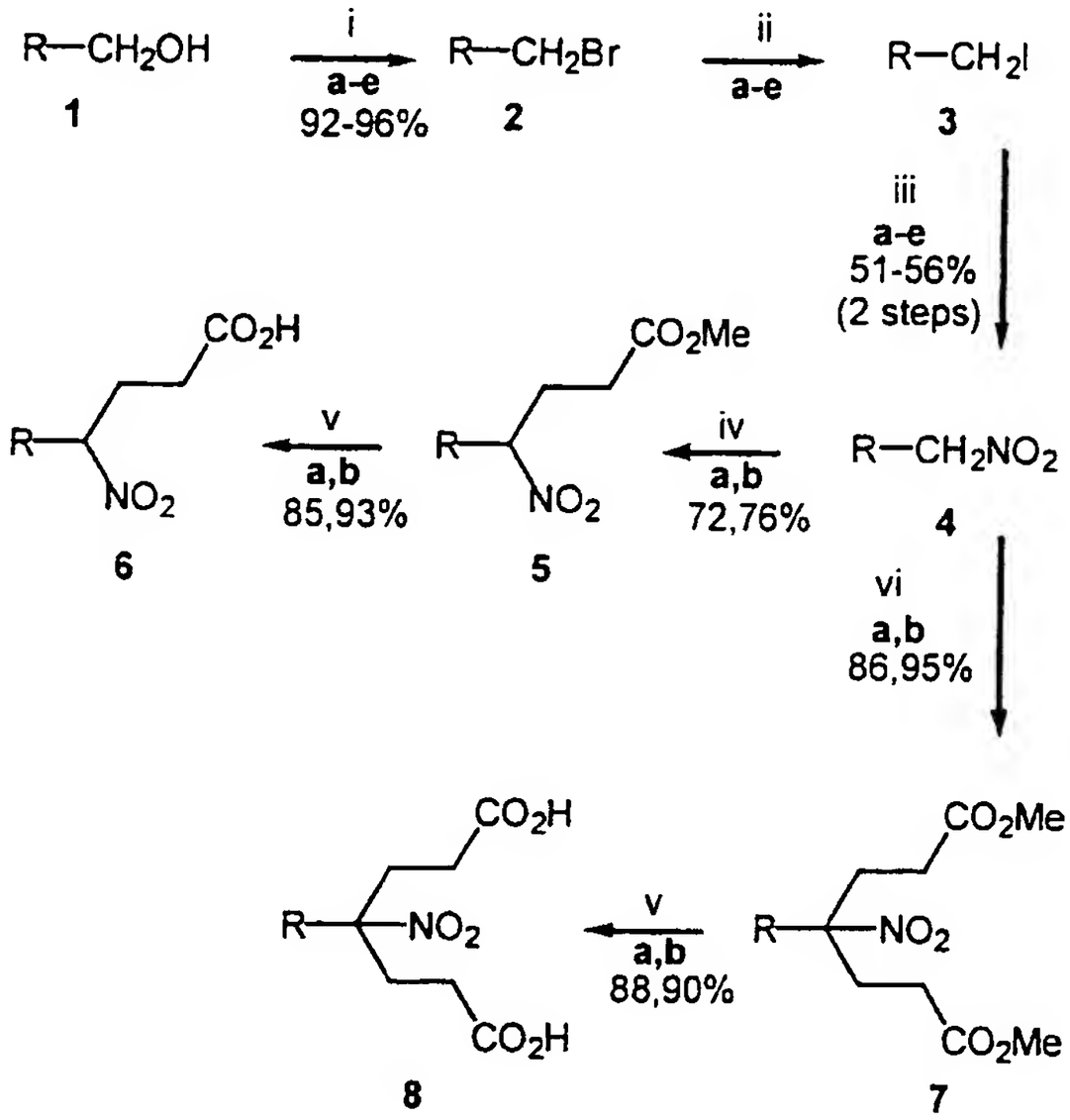
Synthesis of hydroxy and hydroperoxy products of compound 6b was based on Scheme 8. Pure compound 17 was obtained in the yield of 32%. Compound 16 was relatively unstable, but the product with 90% purity was obtained by column chromatography at 0°C, and was used for investigation of its inhibitory effect on 15-LO catalysed oxidation of arachidonic acid.



Scheme 8

(7) Synthesis of polyunsaturated nitroalkanes and nitro-substituted fatty acids.

The polyunsaturated fatty alcohols **1b-e** and the saturated analogue, octadecanol **1a**, are commercially available and were used as starting materials. Their treatment with triphenylphosphine and carbon tetrabromide according to the method of Hayashi *et al.*[23] afforded the corresponding bromides **2a-e**. Short chain bromoalkanes react with silver nitrite to give nitroalkanes[24] but the bromides **2a-e** were inert to such treatment. Instead, they were first treated with sodium iodide to give the iodides **3a-e**, which were used without purification and converted to the nitroalkanes **4a-e**, respectively.



a: R = $CH_3(CH_2)_{16}$-

b: R = (*all*-*Z*)-$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2$-

c: R = (*Z,Z,Z*)-$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6$-

d: R = (*Z,Z,Z*)-$CH_3(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_3$-

e: R = (*all*-*Z*)-$CH_3CH_2(CH{=}CHCH_2)_6CH_2$-

i: PPh_3/CBr_4, CH_2Cl_2, r.t.

ii: NaI, dry acetone, r.t.

iii: $AgNO_2$, Et_2O, r.t.

iv: methyl acrylate, NaOH, Bu_4NI, CH_2Cl_2, reflux

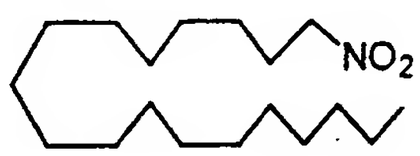
v: LiOH, DME, r.t.

vi: methyl acrylate, DBU, CH_2Cl_2, r.t.

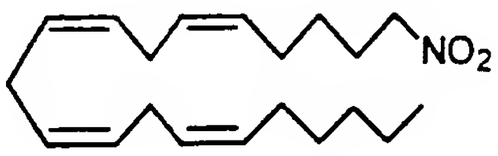
Scheme 9

In order to prepare nitro-substituted fatty acids, a variety of reactions of nitroalkanes were investigated. Carboxylation using the method of Finkbeiner *et al.*[25] was examined. Accordingly reaction of 1-nitropropane with magnesium methyl carbonate afforded 2-nitrobutanoic acid, but 1-nitrooctadecane **(4a)** was recovered unchanged when treated under the same conditions. Apparently the aliphatic chain prevents reaction in the latter case. 1-Nitrooctadecane **(4a)** was treated with butyl lithium then methyl chloroformate[26] to give methyl 2-nitrononadecanoate. However, all attempts to hydrolyse this material to give 2-nitrononadecanoic acid failed, the reactions instead affording the nitroalkane **4a**.

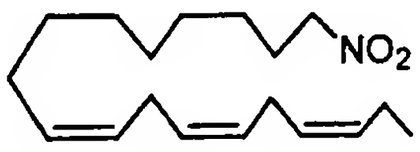
4a



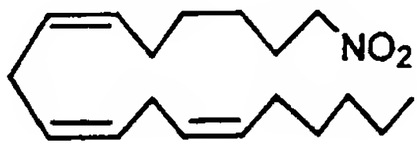
4b



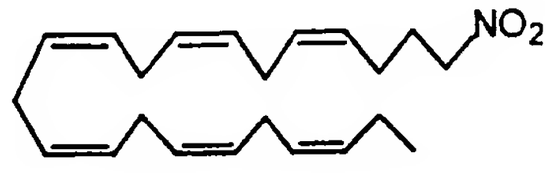
4c



4d



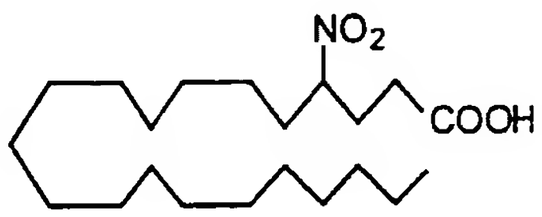
4e



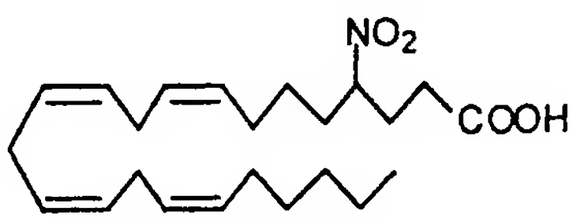
This product (ie nitroalkane **4a**) may be attributed to rapid decarboxylation of the monoanion of 2-nitrononadecanoic acid, since the analogous process has been reported for 2-nitroacetic acid.[27] Given that this decarboxylation would be expected to affect the integrity of 2-nitrocarboxylic acids during physiological studies at near neutral pH, the synthesis of compounds of this type was not further pursued.

The nitroalkane **4a** was inert when treated with butyl lithium and α-haloacetates, indicating that long chain 3-nitrocarboxylates could not be prepared using this approach. However, the nitroalkanes **4a,b** reacted with sodium hydroxide and methyl acrylate[28] in the presence of tetrabutylammonium iodide[29] to give the γ-nitroesters **5a,b**, which were hydrolysed using lithium hydroxide to afford the corresponding nitroacids **6a,b**. Using 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) as the base, in place of sodium hydroxide, the nitroalkanes **4a,b** reacted by sequential Michael additions with methyl acrylate to give the diesters **7a,b**, which hydrolysed to the nitrodiacids **8a,b**.

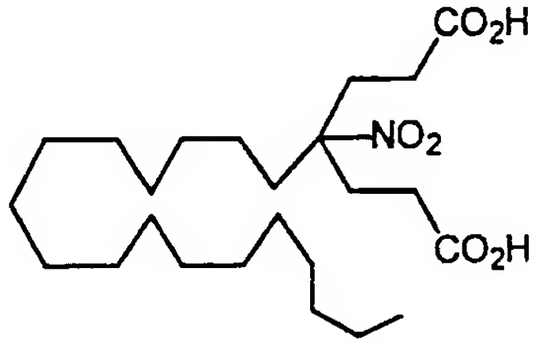
6a



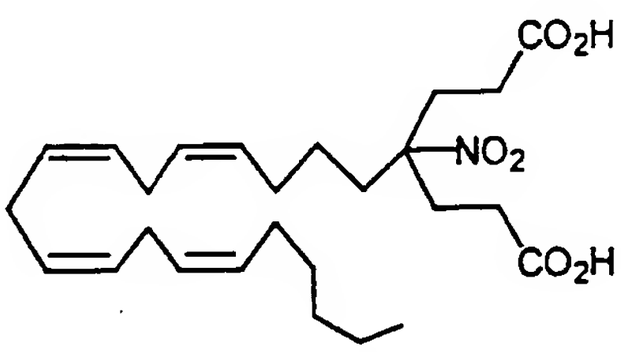
6b



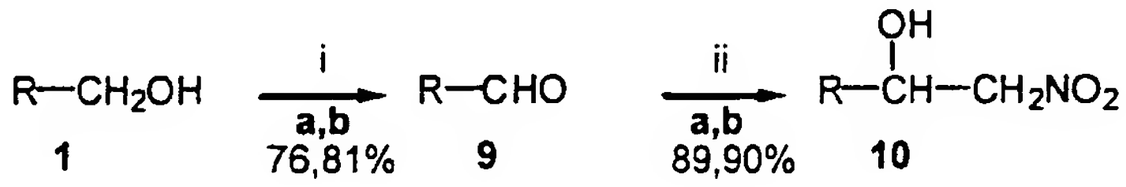
8a



8b



To obtain substituted nitroalkanes, the alcohols **1a,b** were oxidised to the corresponding aldehydes **9a,b** using pyridinium chlorochromate.[30] Henry condensation[31] of these compounds with nitromethane in the presence of Amberlyst A-21[32] afforded the 2-hydroxynitroalkanes **10a,b**, which reacted with methanesulfonyl chloride and triethylamine[33] to give the corresponding α,β-unsaturated nitroalkanes. Unfortunately it was not possible to isolate pure samples of these analogues of α, β-unsaturated fatty acids, because they equilibrated with the corresponding β,γ-unsaturated nitroalkanes and the mixtures of isomers decomposed on chromatography.



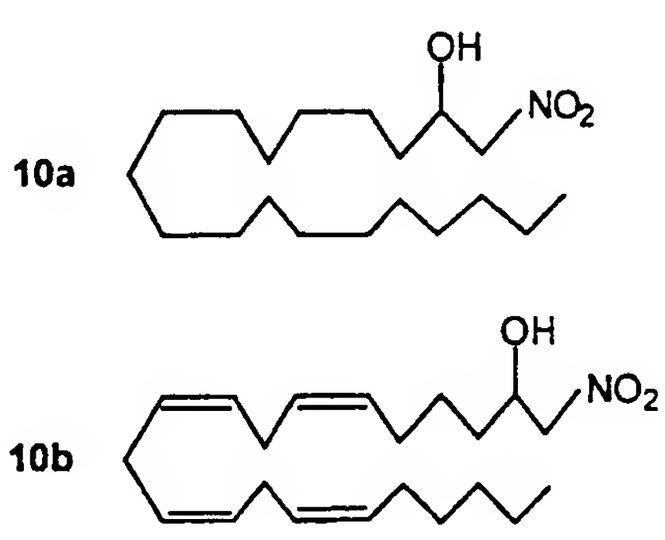
a: R = $CH_3(CH_2)_{16}$-

b: R = (*all-Z*)-$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2$-

i: pyridinium chlorochromate, CH_2Cl_2, r.t.

ii: CH_3NO_2, Amberlyst A-21, Et_2O, reflux

Scheme 10



The reactions described above were carried out under nitrogen and in the dark. After purification the compounds were stored at -30 °C under nitrogen. By taking these precautions there were no complications from isomerisation or

autoxidation of the methylene-interrupted polyenes. Such reactions result in the formation of conjugated dienes and none of the compounds showed absorption at 234 nm which is characteristic of this structural feature.[34]

Experimental

Octadecan-1-ol (**1a**) was obtained from Aldrich Chemical Co. Arachidonyl alcohol (**1b**), linolenyl alcohol (**1c**), gamma linolenyl alcohol (**1d**) and docosahexaenyl alcohol **1e** were purchased from Nu-Chek Prep. Inc. (Elysian, Minnesota, USA).

1-Bromooctadecane (2a); Typical Procedure

Octadecan-1-ol (**1a**) (520 mg, 1.92 mmol) and Ph_3P (550 mg, 2.10 mmol) were dissolved in CH_2Cl_2 (25 mL). The mixture was cooled in an ice bath and CBr_4 (630 mg, 1.90 mmol) was added with stirring. The mixture was allowed to warm to r.t. and was stirred overnight, then it was concentrated under a stream of N_2 and the residue was subjected to flash column chromatography on silica, eluting with hexane, to afford 1-bromooctadecane (**2a**) (605 mg, 96%) as a waxy solid; mp 26-28 °C.

IR (KBr): ν = 2920 (s), 2848 (s), 1468 (s), 1378 (w), 1254 (w), 1144 (m), 720 (m), 658 (s) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.87 (t, 3H, J = 6.7 Hz, C18-H_3), 1.25-1.32 [m, 30H, (C3-17)-H_2)], 1.82-1.85 (m, 2H, C2-H_2), 3.40 (t, 2H, J = 6.8 Hz, C1-H_2).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.2, 28.7, 29.3, 29.9, 30.0, 30.1, 30.1(6), 30.2(3), 32.5, 33.4, 34.6.

MS (EI): *m/z* (%) = 334 (M^+, 8), 332 (M^+, 10), 253 (25), 151 (27), 149 (28), 137 (67), 135 (69), 113 (19), 97 (30), 85 (50), 71 (70), 57 (100).

HRMS: *m/z* calcd for $C_{18}H_{37}Br$ 334.2058 (M^+) and 332.2078 (M^+). Found: 334.2070 and 332.2086.

(*all-Z*)-1-Bromo-5,8,11,14-eicosatetraene (2b)

From arachidonyl alcohol **(1b)** (740 mg, 2.54 mmol), using the procedure described above for preparation of 1-bromooctadecane **(2a)**, (*all-Z*)-1-bromo-5,8,11,14-eicosatetraene **(2b)** (826 mg, 93%) was obtained as a colourless oil.

IR (film): ν = 3012 (s), 2958 (s), 2927 (s), 2856 (s), 1653 (m), 1456 (m), 1394 (m), 1251 (m), 1199 (w), 1041 (m), 915 (w), 807 (w), 715 (s) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.89 (t, 3H, *J* = 6.8 Hz, C20-H_3), 1.29-1.38 (m, 6H, C17-H_2, C18-H_2, C19-H_2), 1.47-1.56 (m, 2H, C3-H_2), 1.83-1.93 (m, 2H, C2-H_2), 2.03-2.14 (m, 4H, C4-H_2, C16-H_2), 2.80-2.83 (m, 6H, C7-H_2, C10-H_2, C13-H_2), 3.42 (t, 2H, *J* = 6.8 Hz, C1-H_2), 5.30-5.41 (m, 8H, C5-H, C6-H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.2, 26.2, 26.9, 27.8, 28.7, 29.9, 32.1, 32.9, 34.3, 128.1, 128.4, 128.7 (2C), 129.0, 129.1, 129.9, 131.1.

MS (EI): *m/z* (%) = 354 (M^+, 5), 352 (M^+, 6), 283 (8), 281 (8), 256 (15), 254 (15), 216 (25), 214 (25), 150 (34), 119 (29), 105 (36), 93 (53), 91 (56), 79 (100), 67 (75).

HRMS: *m/z* calcd for $C_{20}H_{33}Br$ 354.1745 (M^+) and 352.1766 (M^+). Found: 354.1748 and 352.1772.

Anal. Calcd for $C_{20}H_{33}Br$: C, 67.98; H, 9.41. Found: C, 68.05; H, 9.28.

(*Z,Z,Z*)-1-Bromo-9,12,15-octadecatriene (2c)

From linolenyl alcohol **(1c)** (102 mg, 0.39 mmol), using the procedure described above for preparation of 1-bromooctadecane **(2a)**, (*Z,Z,Z*)-1-bromo-9,12,15-octadecatriene **(2c)** (118 mg, 93%) was obtained as a colourless oil.

IR (film): ν = 3001 (s), 2960 (s), 2920 (s), 2850 (s), 1460 (m), 1430 (m), 1395 (w), 1270 (w), 720 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.98 (t, 3H, *J* = 7.5 Hz, C18-H_3), 1.30-1.45 (m, 10H, C3-H_2, C4-H_2, C5-H_2, C6-H_2, C7-H_2), 1.81-1.88 (m, 2H, C2-H_2), 2.03-2.11 (m, 4H, C8-H_2, C17-H_2), 2.80-2.83 (m, 4H, C11-H_2, C14-H_2), 3.41 (t, 2H, *J* = 6.8 Hz, C1-H_2), 5.30-5.42 (m, 6H, C9-H, C10-H, C12-H, C13-H, C15-H, C16-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.9, 21.1, 26.1, 26.2, 27.8, 28.7, 29.3, 29.8, 29.9, 30.2, 33.4, 34.6, 127.7, 128.3, 128.8 (2C), 130.8, 132.5.

MS (EI): *m/z* (%) = 328 (M^+, 14), 326 (M^+, 14), 272 (42), 270 (41), 149 (13), 135 (28), 121 (33), 108 (92), 95 (53), 79 (100), 67 (72), 55 (59).

Anal. Calcd for $C_{18}H_{31}Br$: C, 66.05; H, 9.54. Found: C, 65.82; H, 9.32.

(Z,Z,Z)-1-Bromo-6,9,12-octadecatriene (2d)

From gamma linolenyl alcohol **(1d)** (143 mg, 0.54 mmol), using the procedure described above for preparation of 1-bromooctadecane **(2a)**, (Z,Z,Z)-1-bromo-6,9,12-octadeca-triene **(2d)** (170 mg, 96%) was obtained as a colourless oil.

IR (film): ν = 3002 (s), 2950 (s), 2920 (s), 2850 (s), 1460 (s), 1378 (w), 1260 (w), 715 (m), 648 (m) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ= 0.89 (t, 3H, *J* = 6.8 Hz, C18-H_3), 1.29-1.45 (m, 10H, C3-H_2, C4-H_2, C15-H_2, C16-H_2, C17-H_2), 1.82-1.91 (m, 2H, C2-H_2), 2.02-2.17 (m, 4H, C5-H_2, C14-H_2), 2.79-2.83 (m, 4H, C8-H_2, C11-H_2), 3.40 (t, 2H, *J* = 6.7 Hz, C1-H_2), 5.30-5.41 (m, 6H, C6-H, C7-H, C9-H, C10-H, C12-H, C13-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.2, 26.2, 27.6, 27.8, 28.4, 29.3, 29.9, 32.1, 33.3, 34.5, 128.2, 128.6(5), 128.7(1), 129.0, 130.3, 131.0.

MS (EI): *m/z* (%) = 328 (M^+, 10), 326 (M^+, 8), 230 (49), 228 (50), 150 (66), 135 (15), 121 (25), 107 (32), 93 (59), 79 (100), 67 (95), 55 (64).

HRMS: *m/z* calcd for $C_{18}H_{31}Br$ 328.1589 (M^+) and 326.1609 (M^+). Found: 328.1592 and 326.1611.

(*all*-Z)-1-Bromo-4,7,10,13,16,19-docosahexaene (2e)

From docosahexaenyl alcohol **1e** (201 mg, 0.64 mmol), using the procedure described above for preparation of 1-bromooctadecane **(2a)**, (*all*-Z)-1-bromo-4,7,10,13,16,19-docosahexaene **(2e)** (221 mg, 92%) was obtained as a colourless oil.

IR (film): ν = 3008 (s), 2960 (s), 2928 (s), 2868 (s), 1650 (m), 1434 (s), 1392 (s), 1348 (w), 1322 (w), 1266 (s), 1244 (s), 1068 (m), 1044 (m), 928 (m), 714 (s) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.98 (t, 3H, *J* = 7.5 Hz, C22-H_3), 1.85-2.30 (6H, C2-H_2, C3-H_2, C21-H_2), 2.80-2.90 (m, 10H, C6-H_2, C9-H_2, C12-H_2, C15-H_2, C18-H_2), 3.42 (t, 2H, *J* = 6.6 Hz, C1-H_2), 5.31-5.45 (m, 12H, C4-H, C5-H, C7-H, C8-H, C10-H, C11-H, C13-H, C14-H, C16-H, C17-H, C19-H, C20-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.4, 20.5, 25.5, 25.6, 32.5 33.2, 127.0, 127.8(5), 127.9(4), 128.0(6), 128.1(1) (2C), 128.1(8) (2C), 128.2(4), 128.6, 129.5, 132.0.

MS (EI): *m/z* (%) = 378 (M^+, 10), 376 (M^+, 10), 349 (20), 347 (20), 309 (46), 307 (53), 244 (75), 242 (74), 227 (49), 202 (30), 200 (30), 173 (12), 133 (34), 119 (45), 108 (50), 91 (65), 79 (100), 67 (66).

HRMS: *m/z* calcd for $C_{22}H_{33}Br$ 378.1745 (M^+) and 376.1766 (M^+). Found: 378.1742 and 376.1760.

1-Nitrooctadecane (4a); Typical Procedure

To a solution of 1-bromooctadecane **(2a)** (480 mg, 1.44 mmol) in dry acetone (25 mL) at r.t. was added NaI (430 mg, 2.87 mmol). The mixture was stirred at r.t. overnight, then the solvent was removed *in vacuo*. The residue was mixed with 25 mL of sat. aq sodium bisulfite and the mixture was extracted with Et_2O (3 x 25 mL). The combined extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue (502 mg) was dissolved in anhyd Et_2O and $AgNO_2$ (406 mg, 2.64 mmol) was added. After 3 days of stirring, the mixture was filtered through a bed of celite and the filtrate was evaporated under a stream of dry N_2. The residue was subjected to flash column chromatography on silica (Et_2O/hexane, 5/95) to give crude iodide **3a** (97 mg) and 1-nitrooctadecane **(4a)** (220 mg, 51%) as a white solid; mp 41-42 °C.

IR (film): ν = 2954 (s), 2919 (s), 2850 (s), 1563 (s), 1470 (m), 1385 (w), 1147 (w), 742 (w), 720 (m), 650 (w) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, J = 6.6 Hz, C18-H_3), 1.25-1.34 [m, 30H, (C3-C17)-H_2], 1.96-2.05 (m, 2H, C2-H_2), 4.38 (t, 2H, J = 7.1 Hz, C1-H_2).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 26.7, 28.0, 29.4, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 32.5, 76.3.

MS (EI): m/z (%) = 299 (M^+, <1), 282 (4), 264 (20), 252 (7), 238 (7), 224 (7), 210 (5), 196 (4), 154 (5), 139 (7), 125 (20), 111 (40), 97 (74), 83 (87), 69 (95), 57 (100), 55 (96).

Anal. Calcd for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68. Found: C, 72.33; H, 12.77; N, 4.57.

(*all*-*Z*)-1-Nitro-5,8,11,14-eicosatetraene (4b)

According to the procedure described above for preparation of 1-nitrooctadecane (**4a**), (*all*-*Z*)-1-bromo-5,8,11,14-eicosatetraene (**2b**) (782 mg, 2.21 mmol) gave crude iodide (**3b**) (71 mg) and (*all*-*Z*)-1-nitro-5,8,11,14-eicosatetraene (**4b**) (397 mg, 56%) as a colourless oil.

IR (film): ν = 3013 (s), 2957 (s), 2928 (s), 2857 (s), 1648 (w), 1555 (s), 1457 (m), 1435 (m), 1381 (s), 1267 (w), 1106 (w), 1047 (w), 969 (w), 914 (w), 716 (m) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.89 (t, 3H, *J* = 6.8 Hz, C20-H_3), 1.20-1.51 (m, 8H, C3-C_2, C17-H_2, C18-H_2, C19-H_2), 1.99-2.16 (m, 6H, C2-H_2, C4-H_2, C16-H_2), 2.79-2.86 (m, 6H, C7-H_2, C10-H_2, C13-H_2), 4.39 (t, 2H, *J* = 7.0 Hz, C1-H_2), 5.32-5.43 (m, 8H, C5-H, C6-H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ= 14.6, 23.1, 26.2, 26.7, 26.9, 27.5, 27.8 29.9, 32.1, 76.1, 128.1, 128.4, 128.5, 128.9, 129.2 (2C), 129.6, 131.1.

MS (EI): *m/z* (%) = 319 (M^+, 6), 302 (14), 220 (27), 205 (15), 190 (11), 181 (24), 177 (20), 164 (25), 150 (41), 119 (48), 105 (63), 91 (90), 79 (100), 67 (97), 55 (77).

Anal. Calcd for $C_{20}H_{33}NO_2$: C, 75.19; H, 10.41; N, 4.38. Found: C, 74.92; H, 10.40; N, 4.43.

(*Z*,*Z*,*Z*)-1-Nitro-9,12,15-octadecatriene (4c)

Following the procedure described above for preparation of 1-nitrooctadecane (**4a**), (*Z*,*Z*,*Z*)-1-bromo-9,12,15-octadecatriene (**2c**) (79 mg, 0.24 mmol) gave crude iodide **3c** (12 mg) and (*Z*,*Z*,*Z*)-1-nitro-9,12,15-octadecatriene (**4c**) (37 mg, 53%) as a colourless oil.

IR (film): ν = 3011 (s), 2962 (s), 2929 (s), 2856 (s), 1652 (w), 1554 (s), 1463 (m), 1435 (m), 1383 (m), 1268 (w), 1148 (w), 1069 (w), 968 (m), 912 (w), 724 (m), 614 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.98 (t, 3H, *J* = 7.5 Hz, C18-H_3), 1.25-1.33 (m, 10H, C3-H_2, C4-H_2, C5-H_2, C6-H_2, C7-H_2), 1.97-2.06 (m, 6H, C2-H_2, C8-H_2, C17-H_2), 2.79-2.81 (m, 4H, C11-H_2, C14-H_2), 4.37 (t, 2H, *J* = 7.1 Hz, C1-H_2), 5.36-5.40 (m, 6H, C9-H, C10-H, C12-H, C13-H, C15-H, C16-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.9, 21.1, 26.1, 26.2, 26.8, 27.7, 28.0, 29.4, 29.6, 29.7, 30.1, 76.3, 127.7, 128.4, 128.8, 128.9, 130.7, 132.5.

MS (EI): *m/z* (%) = 293 (M^+, 24), 276 (14), 264 (5), 246 (5), 237 (32), 224 (17), 135 (26), 121 (35), 108 (63), 95 (84), 93 (75), 91 (69), 79 (100), 67 (95).

Anal. Calcd for $C_{18}H_{31}NO_2$: C, 73.67; H, 10.65; N, 4.77. Found: C, 73.69; H, 10.57; N, 4.85.

(*Z,Z,Z*)-1-Nitro-6,9,12-octadecatriene (4d)

Following the procedure described above for preparation of 1-nitrooctadecane **(4a)**, (*Z,Z,Z*)-1-bromo-6,9,12-octadecatriene **(2d)** (122 mg, 0.37 mmol) gave crude iodide **3d** (15 mg) and (*Z,Z,Z*)-1-nitro-6,9,12-octadecatriene **(4d)** (56 mg, 51%) as a colourless oil.

IR (film): ν = 3012 (s), 2956 (s), 2928 (s), 2858 (s), 1652 (m), 1555 (s), 1464 (s), 1435 (s), 1382 (s), 1266 (m), 1159 (w), 1067 (w), 1040 (w), 970 (w), 914 (w), 720 (s), 614 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, *J* = 7.1 Hz, C18-H_3), 1.29-1.43 (m, 10H, C3-H_2, C4-H_2, C15-H_2, C16-H_2, C17-H_2), 2.01-2.08 (m, 6H, C2-H_2, C5-H_2, C14-H_2), 2.78-2.82 (m, 4H, C8-H_2, C11-H_2), 4.38 (t, 2H, *J* = 7.1 Hz, C1-H_2), 5.34-5.40 (m, 6H, C6-H, C7-H, C9-H, C10-H, C12-H, C13-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.2, 26.2, 26.4, 27.4, 27.8, 27.9, 29.4, 29.9, 32.1, 76.2, 128.1, 128.5, 129.0 (2C), 129. 9, 131.0.

MS (EI): *m/z* (%) = 293 (M^+, 31), 276 (25), 258 (12), 246 (4), 222 (7), 195 (72), 150 (36), 137 (18), 105 (25), 91 (84), 81 (80), 80 (79), 79 (100), 67 (82), 55 (60).

Anal. Calcd for $C_{18}H_{31}NO_2$: C, 73.67; H, 10.65; N, 4.77. Found: C, 73.56; H, 10.56; N, 4.74.

(*all-Z*)-1-Nitro-4,7,10,13,16,19-docosahexaene (4e)

Following the procedure described above for preparation of 1-nitrooctadecane **(4a)**, (*all-Z*)-1-bromo-4,7,10,13,16,19-docosahexaene **(2e)** (165 mg, 0.44 mmol) gave crude iodide **3e** (27 mg) and (*all-Z*)-1-nitro-4,7,10,13,16,19-docosahexaene **(4e)** (80 mg, 53%) as a colourless oil.

IR (film): ν = 3014 (s), 2962 (s), 2926 (s), 2873 (s), 2854 (s), 1653 (m), 1554 (s), 1434 (s), 1381 (s), 1352 (m), 1267 (m), 1069 (w), 917 (w), 712 (s), 611 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.98 (t, 3H, *J* = 7.6 Hz, C22-H_3), 2.05-2.23 (m, 6H, C2-H_2, C3-H_2, C21-H_2), 2.78-2.85 (m, 10H, C6-H_2, C9-H_2, C12-H_2, C15-H_2, C18-H_2), 4.38 (t, 2H, *J* = 6.7 Hz, C1-H_2), 5.31-5.47 (m, 12H, C4-H, C5-H, C7-H, C8-H, C10-H, C11-H, C13-H, C14-H, C16-H, C17-H, C19-H, C20-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.8, 21.1, 24.4, 26.1, 26.2, 27.7, 75.4, 127.6, 128.3, 128.4, 128.5(5), 128.6(0), 128.9 (3C), 129.1, 129.2, 130.9, 132.6.

MS (EI): *m/z* (%) = 343 (M^+, 10), 326 (59), 314 (21), 274 (44), 215 (55), 207 (42), 167 (16), 145 (18), 131 (16), 119 (36), 105 (48), 91 (77), 79 (100), 67 (78), 55 (42).

Anal. Calcd for $C_{22}H_{33}NO_2$: C, 76.92; H, 9.68; N, 4.08. Found: C, 76.52; H, 9.87; N, 4.26.

Methyl 4-Nitroheneicosanoate (5a); Typical Procedure

A solution of NaOH (136 mg, 3.4 mmol) and Bu_4NI (158 mg, 0.43 mmol) in water (10 mL) was added to a solution of 1-nitrooctadecane **(4a)** (510 mg, 1.70 mmol) and methyl acrylate (442 mg, 5.13 mmol) in CH_2Cl_2 (10 mL) at r.t. The mixture was stirred and heated at reflux for 24 h, then it was cooled and the layers were separated. The organic phase was washed with water (2 × 25 mL) and dried with Na_2SO_4. The solvent was evaporated and the residue was subjected to flash column chromatography on silica (Et_2O/hexane, 5/95), giving methyl 4-nitroheneicosanoate **(5a)** (498 mg, 76%) as a waxy solid.

IR (Nujol): ν = 2924 (s), 2853 (s), 1744 (s), 1554 (s), 1466 (m), 1439 (m), 1367 (m), 1201 (m), 1175 (m), 1120 (m), 829 (w), 722 (w) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.87 (t, 3H, *J* = 6.7 Hz, C21-H_3), 1.19-1.25 [m, 30H, (C6-C20)-H_2], 1.69-1.78 (m, 1H), 1.92-2.30 (m, 3H), 2.32-2.40 (m, 2H, C2-H_2), 3.69 (s, 3H, OCH_3), 4.50-4.59 (m, 1H, C4-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 26.2, 29.2, 29.5, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 30.5, 32.5, 34.5, 52.5, 88.4, 173.0.

MS (EI): *m/z* (%) = 386 [$(M+1)^+$, 25], 368 (12), 354 (18), 339 (20), 305 (24), 287 (28), 263 (18), 221 (15), 193 (10), 179 (15), 165 (21), 151 (26), 137 (31), 123 (36), 111 (52), 97 (76), 83 (86), 69 (88), 55 (100).

HRMS: *m/z* calcd for $C_{22}H_{44}NO_4$ 386.3270 $(M+H)^+$. Found 386.3275.

Anal. Calcd for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.39; H, 11.53; N, 3.50.

Methyl (*all-Z*)-4-Nitrotricosa-8,11,14,17-tetraenoate (5b)

Following the procedure described above for preparation of methyl 4-nitroheneicosanoate **(5a)**, (*all-Z*)-1-nitro-5,8,11,14-eicosatetraene **(4b)** (650 mg, 2.03 mmol) gave methyl (*all-Z*)-4-nitrotricosa-8,11,14,17-tetraenoate **(5b)** (594 mg, 72%) as a colourless oil.

IR (film): ν = 3065 (w), 3013 (m), 2956 (s), 2930 (s), 2859 (m), 1737(s), 1552 (s), 1439 (m), 1363 (w), 1267 (w), 1263 (w), 1259 (w), 1204 (m), 1178 (m), 981 (w) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, *J* = 6.8 Hz, C23-H_3), 1.24-1.45 (m, 8H, C6-H_2, C20-H_2, C21-H_2, C22-H_2), 1.70-1.81 (m, 1H), 1.91-2.27 (m, 7H), 2.32-2.40 (m, 2H, C2-H_2), 2.73-2.83 (m, 6H, C10-H_2, C13-H_2, C16-H_2), 3.68 (s, 3H, OCH_3), 4.51-4.58 (m, 1H, C4-H), 5.29-5.44 (m, 8H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H, C17-H, C18-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.1, 26.1, 26.2, 26.9, 27.8, 29.2, 29.9, 30.3, 30.5, 32.1, 33.9, 52.5, 88.2, 128.1, 128.4, 128.6, 128.9, 129.2 (2C), 129.6, 131.1, 172.9.

MS (EI): *m/z* (%) = 405 (M^+, 7), 374 (8), 359 (5), 327 (4), 307 (15), 294 (6), 267 (4), 229 (5), 215 (10), 190 (13), 177 (27), 164 (33), 150 (36), 147 (24), 131 (35), 119 (43), 105 (54), 91 (70), 79 (93), 67 (100), 55 (56).

HRMS: *m/z* calcd for $C_{24}H_{39}NO_4$ 405.2879 (M^+). Found 405.2870.

Anal. Calcd for $C_{24}H_{39}NO_4$: C, 71.08; H, 9.69; N, 3.45. Found: C, 71.50; H, 10.03; N, 3.34.

4-Nitroheneicosanoic acid (6a); Typical Procedure

Methyl 4-nitroheneicosanoate **(5a)** (147 mg, 0.38 mmol) was dissolved in 1,2-dimethoxyethane (DME) (2 mL) and sat. aq LiOH solution (2 mL) was added. The mixture was left for 24 h, then it was acidified with dilute HCl (10%, 10 mL) and the mixture was extracted with EtOAc (2 × 10 mL). The extracts were concentrated under a stream of dry N_2 and the residue was subjected to flash column chromatography on silica (Et_2O/hexane, 100/20, then Et_2O/hexane/HOAc, 60/40/1) to afford 4-nitroheneicosanoic acid **(6a)** (121 mg, 85%) as a white solid; mp 55-56 °C.

IR (KBr): ν = 3500-2600 (br), 2955 (m), 2919 (s), 2849 (s), 1698 (s), 1615 (w), 1543 (s), 1467 (m), 1445 (m), 1413 (w), 1360 (w), 1334 (w), 1266 (w), 923 (w), 827 (w), 723 (w), 612 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.87 (t, 3H, *J* = 7.1 Hz, C21-H_3), 1.20-1.28 [m, 30H, (C6-C20)-H_2], 1.69-1.78 (m, 1H), 1.98-2.30 (m, 3H), 2.39-2.48 (m, 2H, C2-H_2), 4.53-4.60 (m, 1H, C4-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 26.2, 28.8, 29.5, 29.8, 29.9, 30.0, 30.1, 30.2(6), 30.3(3), 32.5, 34.4, 88.2, 177.5.

MS (CI): *m/z* = 389.3 $(M+NH_4)^+$.

MS (EI): *m/z* (%) = 354 [$(M-OH)^+$, 2], 323 (19), 321 (19), 305 (17), 287 (14), 263 (12), 236 (5), 221 (9), 193 (10), 179 (15), 165 (15), 151 (17), 137 (20), 125 (25), 110 (73), 97 (100), 83 (64), 69 (64), 55 (73).

HRMS: *m/z* calcd for $C_{21}H_{40}NO_3$ 354.3008 $(M-OH)^+$. Found 354.3006.

Anal. Calcd for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.58; H, 11.08; N, 3.81.

(*all*-*Z*)-4-Nitrotricosa-8,11,14,17-tetraenoic Acid (6b)

Following the procedure described above for preparation of 4-nitroheneicosanoic acid **(6a)**, methyl (*all*-*Z*)-4-nitrotricosa-8,11,14,17-tetraenoate **(5b)** (230 mg, 0.57 mmol) gave (*all*-*Z*)-4-nitrotricosa-8,11,14,17-tetraenoic acid **(6b)** (207 mg, 93%) as a colourless oil.

IR (film): ν = 3611-3317 (br), 3013 (m), 2922 (s), 2852 (m), 2693 (m), 2361 (w), 1714 (s), 1551 (s), 1441 (s), 1379 (m), 1360 (m), 1270 (m), 1071 (m), 969 (w), 916 (m), 844 (m), 824 (w), 720 (m) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.89 (t, 3H, *J* = 7.1 Hz, C23-H_3), 1.27-1.44 (m, 8H, C6-H_2, C20-H_2, C21-H_2, C22-H_2), 1.70-1.82 (m, 1H), 1.93-2.27 (m, 7H), 2.40-2.48 (m, 2H, C2-H_2), 2.78-2.86 (m, 6H, C10-H_2, C13-H_2, C16-H_2), 4.56-4.59 (m, 1H, C4-H), 5.30-5.43 (m, 8H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H, C17-H, C18-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.1, 26.1, 26.2, 26.9, 27.8, 28.9, 29.9, 30.2, 32.1, 33.9, 88.1, 128.1, 128.4, 128.5, 128.9, 129.1, 129.2, 129.7, 131.1, 176.8.

MS (EI): *m/z* (%) = 391 (M^+, 8), 345 (8), 320 (4), 293 (13), 280 (8), 253 (10), 203 (15), 190 (25), 177 (28), 164 (42), 150 (46), 131 (34), 110 (100), 91 (72), 79 (93), 67 (97).

HRMS: *m/z* calcd for $C_{23}H_{37}NO_4$ 391.2723 (M^+). Found 391.2725.

Anal. Calcd for $C_{23}H_{37}NO_4$: C, 70.55; H, 9.52; N, 3.58. Found: C, 70.29; H, 9.86; N, 3.43.

Dimethyl 3-Heptadecyl-3-nitropentane-1,5-dicarboxylate (7a); Typical Procedure

A solution containing 1-nitrooctadecane **(4a)** (50 mg, 0.17 mmol), methyl acrylate (88 mg, 1.02 mmol) and DBU (13 mg, 0.085 mmol) in CH_2Cl_2 (2 mL) was kept at r.t. for 24 h, then it was acidified with HCl (10%, 5 mL) and the mixture was extracted with CH_2Cl_2 (2 x 10 mL). The combined extracts were dried with Na_2SO_4 and concentrated, and the residue was subjected to flash column chromatography on silica (EtOAc/petroleum spirit, 15/85), to give dimethyl 3-heptadecyl-3-nitropentane-1,5-dicarboxylate **(7a)** (76 mg, 95%) as a colourless oil.

IR (film): ν = 2954 (m), 2914 (s), 2849 (s), 1744 (s), 1732 (s), 1537 (s), 1470 (s), 1458 (s) 1439 (s), 1378 (s), 1355 (s), 1319 (s), 1298 (s), 1203 (s), 1180 (s), 1129 (s), 1110 (m), 1071

(m), 1022 (m), 986 (s), 894 (s), 864 (m), 842 (s), 826 (s), 807 (m), 788 (m), 717 (s), 705 (m) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, *J* = 6.8 Hz, C17'-H_3), 1.16-1.25 [m, 30H, (C2'-C16')-H_2], 1.85-1.91 (m, 2H, C1'-H_2), 2.23-2.28 (m, 8H, C2-H_2, C3-H_2, C5-H_2, C6-H_2), 3.69 (s, 6H, OCH_3).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 24.1, 29.1, 29.8, 29.9, 30.0(5), 30.1(2), 30.3, 30.9, 32.5, 36.0, 52.5, 93.3, 173.0.

MS (CI): *m/z* = 489 $(M+NH_4)^+$.

MS (EI): *m/z* (%) = 440 [$(M-OCH_3)^+$, 9], 425 (28), 393 (100), 392 (83), 364 (19), 333 (18), 305 (14), 194 (11), 168 (42), 138 (82), 109 (35), 81 (53).

HRMS: *m/z* calcd for $C_{25}H_{46}NO_5$ 440.3376 $(M-OCH_3)^+$. Found 440.3379.

Anal. Calcd for $C_{26}H_{49}NO_6$: C, 66.21; H, 10.47; N, 2.97. Found: C, 66.63; H, 10.91; N, 2.71.

Dimethyl 3-[(*all*-*Z*)-Nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylate (7b)

Following the procedure described above for synthesis of dimethyl 3-heptadecyl-3-nitropentane-1,5-dicarboxylate **(7a)**, (*all*-*Z*)-1-nitro-5,8,11,14-eicosatetraene **(4b)** (96 mg, 0.30 mmol) gave dimethyl 3-[(*all*-*Z*)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylate **(7b)** (127 mg, 86%) as a colourless oil.

IR (film): ν = 3012 (m), 2955 (m), 2929 (m), 2857 (m), 1742 (s), 1540 (s), 1438 (m), 1379 (w), 1351 (m), 1321 (m), 1260 (m), 1200 (m), 1176 (m), 990 (w), 721 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, *J* = 6.8 Hz, C19'-H_3), 1.25-1.35 (m, 8H, C2'-H_2, C16'-H_2, C17'-H_2, C18'-H_2), 1.86-1.92 (m, 2H, C1'-H_2), 2.03-2.10 (m, 4H, C3'-H_2, C15'-H_2), 2.25-2.37 (m, 8H, C2-H_2, C3-H_2, C5-H_2, C6-H_2), 2.78-2.86 (m, 6H, C6'-H_2, C9'-H_2, C12'-H_2), 3.69 (s, 6H, OCH_3), 5.31-5.43 (m, 8H, C4'-H, C5'-H, C7'-H, C8'-H, C10'-H, C11'-H, C13'-H, C14'-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.6, 23.1, 24.1, 26.2, 27.4, 27.8, 29.1, 29.9, 30.9, 32.1, 35.4, 52.6, 93.2, 128.1, 128.3, 128.5, 128.9, 129.1, 129.2, 129.9, 131.1, 172.9.

MS (EI): *m/z* (%) = 491 (M^+, 16), 460 (72), 444 (50), 429 (28), 413 (70), 393 (42), 381 (28), 357 (36), 333 (14), 301 (50), 207 (26), 181 (32), 164 (34), 150 (40), 133 (40), 121 (50), 106 (71), 93 (86), 80 (78), 79 (100), 67 (98), 55 (60).

HRMS: *m/z* calcd for $C_{28}H_{45}NO_6$ 491.3247 (M^+). Found 491.3247.

Anal. Calcd for $C_{28}H_{45}NO_6$: C, 68.40; H, 9.22; N, 2.85. Found C, 68.77; H, 9.57; N, 2.85.

3-Heptadecyl-3-nitropentane-1,5-dicarboxylic Acid (8a); Typical Procedure

Dimethyl 3-heptadecyl-3-nitropentane-1,5-dicarboxylate **(7a)** (138 mg, 0.29 mmol) was dissolved in DME (2 mL) and sat. aq LiOH solution (2 mL) was added. The mixture was let stand for 22 h, then it was acidified with dilute HCl (10%, 10 mL) and extracted with EtOAc (2 x 10 mL). The extracts were concentrated under a stream of dry N_2 and the residue was subjected to flash column chromatography on silica (EtOAc/petroleum spirit, 15/85) to afford 3-heptadecyl-3-nitropentane-1,5-dicarboxylic acid **(8a)** (93 mg, 90%) as a white solid; mp 102 °C.

IR (Nujol): ν = 3600-2700 (br), 2919 (s), 2852 (s), 1740 (s), 1700 (w), 1652 (w), 1534 (s), 1467 (m), 1454 (m), 1428 (m), 1353 (w), 1323 (m), 1282 (m), 1267 (w), 1234 (m), 1224 (s), 894 (w), 834 (w), 814 (w), 721 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, J = 6.8 Hz, C17'-H_3), 1.17-1.30 [m, 30H, (C2'-C16')-H_2], 1.85-1.91 (m, 2H, C1'-H_2), 2.26-2.40 (m, 8H, C1-H_2, C2-H_2, C4-H_2, C5-H_2).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 23.9, 29.1, 29.4, 29.8, 29.9(0), 29.9(3), 30.0, 30.1, 30.2, 30.3, 32.5, 37.7, 93.8, 179.2.

MS (CI): *m/z* = 461 $(M+NH_4)^+$.

MS (EI): *m/z* (%) = 426 [$(M-OH)^+$, 1], 397 (3), 379 (68), 377 (70), 359 (56), 350 (28), 332 (42), 323 (56), 305 (30), 168 (77), 157 (100), 138 (56), 129 (56), 111 (58), 97 (58), 81 (58), 71 (64), 57 (68).

HRMS: *m/z* calcd for $C_{24}H_{44}NO_5$ 426.3219 $(M-OH)^+$. Found 426.3229.

Anal. Calcd for $C_{24}H_{45}NO_6$: C, 64.98; H, 10.22; N, 3.16. Found: C, 64.55; H, 10.69; N, 2.81.

3-[(*all-Z*)-Nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic Acid (8b)

Following the procedure described above for synthesis of 3-heptadecyl-3-nitropentane-1,5-dicarboxylic acid **(8a)**, dimethyl 3-[(*all-Z*)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylate **(7b)** (110 mg, 0.22 mmol) gave 3-[(*all-Z*)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic acid **(8b)** (90 mg, 88%) as a white solid; mp 50-51 °C.

IR (film): ν = 3400-2300 (br), 3013 (s), 2955 (s), 2927 (s), 2855 (s), 2734 (m), 2630 (m), 1742 (s), 1714 (s), 1538 (s), 1439 (s), 1353 (s), 1321 (s), 1291 (s), 1231 (s), 1068 (m), 989 (m), 918 (s), 833 (s), 807 (m), 803 (m), 732 (m), 678 (m), 622 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.89 (t, 3H, *J* = 6.9 Hz, C19'-H_3), 1.21-1.38 (m, 8H, C2'-H_2, C16'-H_2, C17'-H_2, C18'-H_2), 1.85-1.91 (m, 2H, C1'-H_2), 2.03-2.09 (m, 4H, C3'-H_2, C15'-H_2), 2.26-2.38 (m, 8H, C1-H_2, C2-H_2, C4-H_2, C5-H_2), 2.77-2.86 (m, 6H, C6'-H_2, C9'-H_2, C12'-H_2), 5.25-5.47 (m, 8H, C4'-H, C5'-H, C7'-H, C8'-H, C10'-H, C11'-H, C13'-H, C14'-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.1, 23.9, 26.2, 27.2, 27.8, 29.2, 29.7, 29.9, 32.1, 36.4, 93.4, 128.1, 128.3, 128.4, 128.9 (2C), 129.2, 130.0, 131.1, 178.8.

MS (EI): *m/z* (%) = 463 (M^+, 16), 446 (4), 416 (24), 397 (6), 365 (4), 343 (8), 305 (6), 278 (10), 245 (12), 231 (12), 217 (14), 203 (22), 192 (20), 177 (56), 164 (42), 157 (38), 145 (30), 138 (50), 119 (54), 106 (72), 93 (82), 91 (76), 80 (72), 79 (100), 69 (46), 67 (98), 55 (64).

HRMS: *m/z* calcd for $C_{26}H_{41}NO_6$ 463.2934 (M^+). Found 463.2942.

Anal. Calcd for $C_{26}H_{41}NO_6$: C, 67.36; H, 8.91; N, 3.02. Found: C, 67.51; H, 9.23; N, 2.92.

Octadecanal (9a); Typical Procedure

PCC (6 g, 27.83 mmol) was suspended in CH_2Cl_2 (30 mL), and octadecan-1-ol **(1a)** (5.02 g, 18.57 mmol) in CH_2Cl_2 (15 mL) was then rapidly added at r.t. The solution became briefly homogeneous before the deposition of the black insoluble reduced reagent. After 2 h, the black mixture was diluted with five volumes of anhyd Et_2O, the solvent was decanted, and the black solid was washed twice with Et_2O. The crude product was isolated by filtration of the organic solutions through Florisil and concentration of the filtrate under reduced pressure. Purification by flash column

chromatography on silica (Et_2O/hexane, 4/96) gave octadecanal **(9a)** (4.02 g, 81%) as a white solid; mp 43-44 °C.

IR (Nujol): ν = 2960 (s), 2910 (s), 2850 (s), 2705 (w), 1730 (s), 1460 (s), 1375 (s), 720 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (t, 3H, *J* = 6.4 Hz, C18-H_2), 1.28 [m, 28H, (C4-C17)-H_2], 1.58-1.65 (m, 2H, C3-H_2), 2.42 (t, 2H, *J* = 7.3 Hz, C2-H_2), 9.76 (s, 1H, CHO).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 22.7, 23.3, 29.7, 29.9, 30.0, 30.1, 30.3, 32.5, 44.5, 203.6.

MS (EI): *m/z* (%) = 268 (M^+, 4), 250 (34) , 224 (17), 222 (18), 208 (6), 194 (10), 182 (8), 166 (8), 152 (10), 137 (20), 124 (30), 110 (42), 96 (74), 82 (100), 71 (82), 69 (69), 57 (53), 55 (57).

HRMS: *m/z* calcd for $C_{18}H_{36}O$ 268.2766 (M^+). Found: 268.2765.

Anal. Calcd for $C_{18}H_{36}O$: C, 80.53; H, 13.51. Found: 80.46, H, 13.49.

(*all-Z*)-Eicosa-5,8,11,14-tetraenal (9b)

According to the procedure described above for preparation of octadecanal **(9a)**, arachidonyl alcohol **(1b)** (402 mg, 1.38 mmol) gave (*all-Z*)-eicosa-5,8,11,14-tetraenal **(9b)** (303 mg, 76%) as a colourless oil.

IR (film): ν = 3005 (s), 2960 (s), 2910 (s), 2850 (s), 1730 (s), 1460 (w), 1390 (w), 1160 (w), 920 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ= 0.89 (t, 3H, *J* = 6.8 Hz, C20-H_3), 1.28-1.34 (m, 6H, C17-H_2, C18-H_2, C19-H_2), 1.69-1.74 (m, 2H, C3-H_2), 2.04-2.14 (m, 4H, C4-H_2, C16-H_2), 2.42-2.45 (m, 2H, C2-H_2), 2.79-2.85 (m, 6H, C7-H_2, C10-H_2, C13-H_2), 5.34-5.40 (m, 8H, C5-H, C6-H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H), 9.78 (s, 1H, CHO).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.5, 22.3, 23.0, 26.1, 26.9, 27.6, 29.7, 31.9, 43.7, 127.9, 128.2, 128.4, 128.7, 129.0, 129.2, 129.5, 130.9, 202.9.

MS (EI): *m/z* (%) = 288 (M^+, <1), 244 (1), 234 (1), 217 (2), 203 (3), 177 (9), 164 (13), 150 (30), 131 (12), 119 (19), 106 (59), 93 (56), 91 (64), 80 (77), 79 (100), 67 (93), 55 (43).

HRMS: *m/z* calcd for $C_{20}H_{32}O$ 288.2453 (M^+). Found: 288.2449.

Anal. Calcd for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 83.28; H, 11.12.

1-Nitrononadecan-2-ol (10a); Typical Procedure

To a solution of octadecanal **(9a)** (2.22 g, 8.28 mmol) and nitromethane (1.52 g, 24.90 mmol) in anhyd Et_2O (10 mL), Amberlyst A-21 (1.2 g) was added at r.t. The mixture was stirred and heated at reflux for 48 h. After removal of the Amberlyst A-21 by filtration, the filtrate was concentrated under reduced pressure. Flash column chromatography of the residue (EtOAc/petroleum spirit, 5/95) gave 1-nitrononadecan-2-ol **(10a)** (2.41 g, 89%) as a white solid; mp 55-56 °C.

IR (Nujol): ν = 3500-3300 (br), 2960 (s), 2910 (s), 2850 (s), 1550 (m), 1460 (s), 1375 (s), 720 (w) cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ = 0.86-0.90 (m, 3H, C19-H_3), 1.26 [m, 30H, (C4-C18)-H_2], 1.43-1.55 (m, 2H, C3-H_2), 2.22-2.43 (bs, 1H, OH), 4.28-4.46 (m, 3H, C1-H_2, C2-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.7, 23.3, 25.7, 29.8(8), 29.9(2), 30.0, 30.1, 30.2, 30.3, 32.5, 34.3, 69.2, 81.2.

MS (CI): *m/z* = 347 $(M+NH_4)^+$.

MS (EI): *m/z* (%) = 311 [$(M-H_2O)^+$, 3], 294 (32), 282 (9), 276 (27), 267 (31), 250 (34), 240 (6), 222 (15), 208 (8), 194 (9), 179 (7), 165 (10), 151 (16), 137 (37), 123 (62), 109 (85), 97 (95), 95 (100), 83 (100), 69 (88), 57 (92), 55 (92).

HRMS: *m/z* calcd for $C_{19}H_{37}NO_2$ 311.2824 $(M-H_2O)^+$. Found: 311.2831.

Anal. Calcd for $C_{19}H_{39}NO_3$: C, 69.25; H, 11.93, N, 4.25. Found: C, 69.54, H, 12.18, N, 4.13.

(*all*-*Z*)-1-Nitroheneicosa-6,9,12,15-tetraen-2-ol (10b)

According to the procedure described above for synthesis of 1-nitrononadecan-2-ol **(10a)**, (*all*-*Z*)-eicosa-5,8,11,14-tetraenal **(9b)** (220 mg, 0.76 mmol) gave (*all*-*Z*)-1-nitroheneicosa-6,9,12,15-tetraen-2-ol **(10b)** (240 mg, 90%) as a colourless oil.

IR (film): ν = 3600-3300 (br), 3005 (s), 2960 (s), 2910 (s), 2850 (s), 1650 (w), 1550 (s), 1460 (m), 1440 (m), 1380 (s), 1260 (w), 910 (w), 720 (s) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.87-0.91 (m, 3H, C21-H_3), 1.27-1.39 (m, 6H, C18-H_2, C19-H_2, C20-H_2), 1.50-1.56 (m, 4H, C3-H_2, C4-H_2), 2.02-2.16 (m, 4H, C5-H_2, C17-H_2), 2.40-2.60 (bs, 1H, OH), 2.80-2.86 (m, 6H, C8-H_2, C11-H_2, C14-H_2), 4.29-4.45 (m, 3H, C1-H_2, C2-H), 5.30-5.45 (m, 8H, C6-H, C7-H, C9-H, C10-H, C12-H, C13-H, C15-H, C16-H).

^{13}C NMR ($CDCl_3$, 300 MHz): δ = 14.5, 23.0, 25.5, 26.0, 27.1, 27.6, 29.7, 31.9, 33.5, 68.9, 81.0, 127.9, 128.2, 128.5, 128.6, 129.0, 129.1, 129.5, 130.9.

MS (EI): *m/z* (%) = 349 (M^+, <1), 314 (1), 251 (2), 234 (1), 217 (2), 203 (3), 177 (6), 164 (10), 150 (24), 131 (13), 119 (21), 106 (43), 93 (57), 91 (71), 79 (100), 67 (92), 55 (48).

HRMS: *m/z* calcd for $C_{21}H_{35}NO_3$ 349.2617 (M^+). Found: 349.2614.

Anal. Calcd for $C_{21}H_{35}NO_3$: C, 72.17; H, 10.09, N, 4.01. Found: C, 72.25, H, 9.91; N, 3.64.

B. DETERMINATION OF BIOLOGICAL ACTIVITY OF NITRO COMPOUNDS [4a (Lx1); 4b (Lx4); 6a (Lx6); 6b (Lx7); 8a (Lx8) and 8b (Lx9)]

(1) Investigation of 15-LO, 5-LO and 12-LO catalysed oxidation of the nitro compounds (4a, 4b, 6a, 6b, 8a and 8b;Table 1)

It has been suggested the various hydroxy and hydroperoxy fatty acid derivatives (such as 15-HETE and 15 HPETE) have inhibitory effects on lipoxygenase enzymes. [35] Based on this consideration, 5-LO, 12-LO and 15-LO catalysed oxidation of the nitro compounds (4a, 4b, 6a, 6b, 8a and 8b) was investigated. Each of the nitro compounds was treated with 15-LO in pH 9.0 buffer (or 5-LO in pH 6.3 buffer and 12-LO in pH 7.4 buffer), and the formation of 15-hydroperoxy derivatives (or 5-hydroperoxy or 12-hydroperoxy derivatives) over time was monitored by UV spectroscopy at 234nm. The result shows that, among the nitro compounds, compound 6b was the only one that underwent lipoxygenase catalysed oxidation. It served as a substrate for both 15-LO and 12-LO, but not for 5-LO.

(2) The effect of nitro compounds 4a (Lx1), 4b (Lx4), 6a (Lx6), 6b (Lx7), 8a (Lx8) and 8b (Lx9) on 15-LO, 5-LO and 12-LO catalysed oxidation of arachidonic acid

The result from the preliminary experiment is summarised in Table 2. It shows that compound 8a has an inhibitory effect on 15-LO but not on 5-LO, while compound 6a displays complementary activity inhibiting 5-LO but not 15-LO. Neither 8a nor 6a inhibits 12-LO. Compound 8b appears to have a significant inhibitory effect on 12-LO catalysed oxidation of arachidonic acid, giving a relatively long lagtime at the early stage of arachidonic acid oxidation.

(3) The inhibitory effect of 15-hydroperoxy and 15-hydroxy derivatives from compound 6b on 15-LO catalysed oxidation of arachidonic acid

An enzyme assay shows that these two compounds did have inhibitory effect on 15-LO catalysed oxidation of arachidonic acid, giving IC_{50} values of 50μM for 15-hydroperoxy derivative of 6b and 120 μM for 15-hydroxy derivative of 6b.

(4) Determination of K_m and V_{max} for 15-LO catalysed oxidation of compound 6b, and inhibitor constant of compound 8a on 15-LO catalysed oxidation of arachidonic acid

The Michaelis constant K_m and the value of V_{max} for 15-LO catalysed oxidation of compound 6b were measured and calculated based on the Lineweaver Burke equation, with K_m as 8.4 μM and V_{max} as 24.48 μM/min.

The inhibitor constant (K_i or K_I) of compound 8a was also determined. The graph of 1/v vs 1/[s] with varying concentrations of compound 8a indicates that the inhibition is of the mixed inhibition pattern as shown in the following scheme. Thus the K_i and K_I values in the scheme were calculated giving the result of 27.42 μM for K_i and 55.15 μM for K_I.

Table 2 Effect of nitro compounds on oxidation of arachidonic acid (AA) catalysed by 15-LO, 5-LO or 12-LO

Compounds	Effect on 15-LO catalysed oxidation of AA	Effect on 5-LO catalysed oxidation of AA	Effect on 12-LO catalysed oxidation of AA
Lx1	Nil	Nil	Nil
Lx4	Nil	Nil	Nil
Lx6	Activatory	Inhibitory IC_{50}=60μM	Activatory
Lx8	Inhibitory K_i = 27.42 μM K_I = 55.15 μM	Nil	Activatory
Lx7	Substrate K_m = 8.4 μM V_{max}= 24.48 μM/min	Activatory	Substrate
Lx9	Nil	Activatory	Inhibitory
Lx2	Nil	Nd	Nd
Lx3	Nil	Nd	Nd
Lx5	Nil	Nd	Nd

Nd = Not done

C. ANTI-TUMOUR EFFECTS OF NITRO COMPOUNDS

(1) Prostate Cancer

Prostate cancer is the most often diagnosed non-skin cancer and second largest cause of cancer related death in men in the United States[36]. Prostate cancer usually commences as an androgen-dependent cancer which responds well to treatments such as hormone ablation therapy. However, the cancer can progress to an androgen-independent form which is usually fatal[37]. The prognosis for prostate cancer is so poor that the Urological Society of Australasia has stated that there is no point undertaking population screening until there are viable treatments available[38]. Therefore, there is an urgent need for new therapies to treat androgen-independent prostate cancer; ideally therapies which induce apoptosis in androgen-independent prostate cancer cells.

As humans are one of the few species to get prostate cancer, whole animal work has been limited. This situation has recently changed with the release of a mouse genetically-engineered to develop prostate cancer[39]. However, most research work on prostate cancer so far has been done using human-derived tissue culture cell lines. The three most commonly used cell lines are LNCaP, DU145 and PC3 which were derived from humans with metastases to lymph node, brain and bone respectively. LNCaP cells are androgen sensitive in that the addition of androgens can cause a biological response, eg growth modulation[40], but they are not androgen-dependent as they do not die following withdrawal of androgen and can continue to proliferate in the absence of androgen. DU145 and PC3 cells are androgen-independent as they do not respond to the addition or withdrawal of androgen. DU145 and PC3 cells are generally more resistant to inducers of apoptosis than LNCaP. For example, the phorbol ester, phorbol 12-myristate 13-acetate, induces apoptosis in LNCaP but not DU145 cells[41]. The increased resistance of androgen-independent cell lines mirrors the clinical situation where androgen-independent cancer is resistant to treatment and is usually fatal.

Although the aetiology of prostate cancer is still poorly understood, there is evidence that dietary fat intake can influence prostate cancer risk. While intake of

high levels of the n-6 fatty acid, arachidonic acid (20:4n-6), promotes the growth of prostate and breast cancers, increased intake of n-3 fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), found in abundance in fish oils, reduces the risk of these cancers[42]. An analysis of the ratio of n-3:n-6 levels in the serum of patients with prostate cancer and benign prostate hyperplasia, and age-matched controls, has revealed that the patients have a lower n-3:n-6 fatty acids compared to controls[43]. Another study has found that reduced prostate cancer risk is associated with increased levels of 20:5n-3 and 22:6n-3 esterified in phosphatidylcholine in cellular membranes[44]. These studies therefore suggest that the polyunsaturated fatty acids (PUFA) or their metabolites are important regulators of prostate cancer development and growth.

Fatty acids are essential components of cellular membranes and are an important source of fuel. Furthermore, fatty acids, especially PUFA such as 20:4n-6, 20:5n-3 and 22:6n-3, are biologically active when added exogenously to a variety of cell-types. The actions of these PUFA range from stimulation of neutrophil responses to inhibition of cell-cell communication via gap junctions[45,46]. n-6 and n-3 PUFA have also been demonstrated to stimulate the activities of protein kinase C and MAP kinases such as the extracellular signal-regulated protein kinase, c-jun N-terminal kinase and p38, and to cause an increase in intracellular Ca^{2+} concentration[47-50]. Inhibitor studies have indicated that some of the actions of the PUFA are mediated by these intracellular signalling molecules[50]. The actions of the PUFA can also be mediated by their metabolites derived from the 5-, 12- and 15-lipoxygenases (LOX), and cyclooxygenases (COX) 1 and 2. For example, 5-LOX-derived eicosanoids, 5-oxo eicosatetraenoic acid and leukotriene B4 are potent activators of neutrophils and eosinophils[51]. PUFA can also influence cellular responses by being incorporated into membrane phospholipids which serve as substrates for phospolipases A_2, C and D, thereby giving rise to important lipid-based second messenger molecules such as non-esterified fatty acids, diacylglycerol, phosphatidic acid and lysophosphatidic acid.

The reasons for the above described antithetic actions of n-3 and n-6 PUFA on prostate cancer risk have not been elucidated. Since 20:5n-3 PUFA compete against 20:4n-6 as substrates for 5-LOX, it is possible that a reduction in eicosanoids derived from 20:4n-6 in the presence of 20:5n-3 may have an impact on the survival of prostate cancer cells. Similarly, such competition between n-3 and n-6 PUFA for metabolism by COX may also be relevant. If this is correct, inhibition of the 5-LOX or COX will result in the death of prostate cancer cells. Interestingly, it has recently been demonstrated that apoptosis can indeed be induced in prostate cancer cell lines using inhibitors of 5-LOX such as NDGA and MK886[52,53] or COX such as ibuprofen[54]. For 5-LOX to be active, it must be bound to 5-LOX activating protein (FLAP)[55]. MK886 works by binding to FLAP while NDGA is a broad inhibitor of lipoxygenase activity. The effect of 5-LOX inhibitors can be reversed by the addition of 5-hydroxyeicosatetraenoic acid, but not by 12- or 15- hydroxyeicosatetraenoic acid[53]. This provides strong evidence that (a) product(s) of 5-LOX is (are) needed for the survival of prostate cancer cells. The apoptosis-inducing action of 5-LOX inhibitors is not restricted to prostate cancer cell lines, as inhibition of 5-LOX has also been shown to induce apoptosis in a number of lung cancer cell lines and in *in vivo* models of lung cancer [56]. These results suggest that 5-LOX may be a target for anti-cancer drugs. Besides the Lx compounds described above, we have also previously synthesized β-oxa and β-thia fatty acids (MP) (International Patent Specification No. PCT/AU95/00677) and fatty acid-amino acid conjugates (PT) (International Patent Specification No. PCT/AU95/00717) (Table 3). Our investigations with some of these compounds have revealed that a number of them are strong inhibitors of purified 5-LOX and 5-LOX catalysed production of leukotriene B4 in neutrophils but not of COX in these cells. Others alter the LOX differently. Thus an examination of the ability of these PUFA to kill cancer cells was undertaken.

Table 3. Synthetic fatty acids and related compounds.

β-oxa PUFA	β-and γ-thia PUFA	Amino acid-conjugated PUFA
MP1 (β-oxa-23:0)	MP1 (β-thia 23:0)	PT7 (18:3n-6-Gly)
MP4 (β-oxa-21:3n-6)	MP9 (β-thia-21:3n-6)	PT8 (18:3n-6-Asp)
MP5 (β-oxa-21:3n-3)	MP10 (β-thia-21:3n-3)	PT9 (18:3n-3-Gly)
MP7 (β-oxa-21:4n-3)	MP8 (β-thia-23:4n-6)	PT10 (18:3n-3-Asp)
MP3 (β-oxa-23:4n-6)	MP12 (γ-thia-22:3n-6)	PT1 (20:4n-6-Gly)
MP6 (β-oxa-25:6n-3)	MP13 (γ-thia-22:3n-3)	PT2 (20:4n-6-Asp)
	MP11 (γ-thia 24:4n-6)	PT3 (20:5n-3-Gly)
	MP14 (γ-thia-25:6n-3)	PT4 (20:5n-3-Asp)
	MP15 (α-CH_2CO_2H-β-thia 23:4n-6)	PT5 (22:6n-3-Gly)
	MP16 (15-OOC$(CH_3)_2$ $OCH_3$20:4n-6)	PT6 (22:6n-3-Asp)
	MP17 (15'-OOC$(CH_3)_2$ OCH_3 β-oxa 23:4n-6)	

Protected hydroperoxy PUFA	Nitroso-compounds
MP16 (15-OOC $(CH_3)_2$ OCH_3 20:4n-6)	LX1 (19:0-NO_2)
MP17 (15'-OOC $(CH_3)_2$ OCH_3 β-oxa-23:4n-6)	LX2 (19:3 n-3-NO_2)
Hydroxyβ-oxa-PUFA	LX3 (19:3 n-6-NO_2)
TR1 (16-OH β-oxa-21:3n-6)	LX4 (21:4 n-6-NO_2)
TR2 (16-OH β-oxa-21:3n-3)	LX5 (23:6 n-3-NO_2)
	LX6 (21:0 -γ-NO_2)
	LX7 (23:4 n-6γ-NO_2)
	LX8 (γ-COOH-19:0-NO_2)
	LX9 (γ-COOH-21:4n-6-NO_2)

Lx compounds were tested for activity against two androgen insensitive prostate cancer tumour cell lines, DU145(~~liver~~ brain metastases) and PC3 (~~brain~~ s metastases). The compounds showed anti tumour effects (Figs 1,2,3). Fig 1 shows the survival of DU145 of Lx compound. Survival was measusred using the MTS cell proliferation assay.

Various concentrations for varying amounts of time of Lx compounds were added to either DU145 or PC3 tumour cells in culture. Viability/death of tumour cells was measured by a standard colourimetric assay. The results in Fig 2 show both the concentration and time related effects of Lx6 on DU145 tumour cells. Using a concentration of 15μM, killing of all the tumour cell population occurred after 24 hours of culture. It is also evident from the results (Fig 2) that the saturated nitro compound Lx1 (19:0-NO_2) is not active and neither is MP2 (β-thia 23:0), a saturated β-oxa fatty acid. Using the PC3 tumour cell line, similar results were found (Fig 3).

In a further screening test, the human prostate cell line, DU145 cells (~~liver~~ b metastases), was treated for 24 hrs with 20μM Fatty Acid and cell survival was

measured using the MTS cell proliferation assay. This assay uses colourimetric measurement of substrate conversion to formazan which occurs only in the presence of NADH in a metabolically active cell. The results are presented in Fig 4. There was varied anti-tumour activity amongst the different types of PUFA. Of the MP compounds, MP6 (β-oxa-25:6n-3), MP9 (β-thia-21:3n-6), MP10 (β-thia-21:3n-3), MP12 (γ-thia-22:3n-6) and MP17 (15'-$OOC[CH_3]_2OCH_3$ β-oxa-23:4n-6) were the most active in killing DU145 cells. In addition, MP3 (β-oxa-23:4n-6), MP8 (β-thia-23:4n-6) and MP13 (β-thia-22:3n-3) were also highly active. In the PT series, PT5 (22:6n-3 Gly) showed some activity. These studies show that the anti-cancer activity of the PUFA is dependent on their structure. Using the PC3 cell line (~~brain~~ spine metastases), similar results were found.

To see whether the method by which the fatty acids killed tumour cells was by apoptosis, we measured the activation of caspases. DU145 cells were treated with PUFA and incubated for 24h (for the proliferation assay), 4h (for the caspase assay) and for 18h (for PARP cleavage).

Proliferation was quantitated by MTS assay. Caspases 3 and 7 were assayed by a fluorometric assay using a DVED substrate. PARP cleavage was measured by Western blots using an anti PARP antibody. The results showed that, under conditions where the PUFA, MP3 and MP5 caused marked inhibition of cell proliferation (Fig 5A), there was activation of caspases in association with the cleavage of PARP (Figs 5B and 5C). MP3 and MP5, but not MP2, induce apoptosis in DU145 cells.

(2) **Breast Cancer**

Breast cancer is the most commonly diagnosed cancer and the main cause of cancer-related death in women in Australia[57]. This is in contrast to Japan, where breast cancer is rare even though Japanese women living in western countries have the same incidence rates as western women[58]. This has given rise to the hypothesis that environmental factors can affect breast cancer risk. One environmental

difference which has generated a lot of interest is diet. The Japanese diet contains more fish than the western diet and fish contains high levels of n-3 fatty acids.

There has been some research into the effect of n-3 fatty acids on breast cancer. While intake of high levels of the n-6 fatty acid, arachidonic acid (20:4n-6), promotes the growth of prostate and breast cancers, increased intake of n-3 fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), found in abundance in fish oils, reduces the risk of these cancers[59]. n-3 fatty acids have also been shown to inhibit metastasis of human breast cancer xenografts in mice whereas n-6 fatty acids promoted metastasis[60]. These studies suggest that the polyunsaturated fatty acids (PUFA) or their metabolites are important regulators of breast cancer development and growth.

As has been discussed for prostate cancer, it is possible that a reduction in eicosanoids derived from 20:4n-6 in the presence of n-3 PUFA may have an impact on the survival of breast cancer cells. Similarly, such competition between n-3 and n-6 PUFA for metabolism by COX may also be relevant. If this is correct, then interference with metabolism of 20:4n-6 by LOX or COX will result in the death of breast cancer cells[61].

Interestingly, it has been reported that breast cancer cells are dependent on 5-LOX and 12-LOX but in different ways. Addition of 5-LOX to MCF-7 breast cancer cells inhibits their growth and the 5-LOX inhibitor MK886 can reverse 5-LOX growth inhibition[62]. Therefore, the 5-LOX derived metabolites must be anti-proliferative. In contrast, expression of 12-LOX in MCF-7 cells enhances growth[63]. Furthermore, breast cancer biopsies and cell lines have increased expression of 12-LOX mRNA compared to benign breast tissue and cell lines[64]. Therefore, products of 12-LOX must stimulate breast cancer growth. These results suggest that activation of 5-LOX and inhibition of 12-LOX may be a means of treating breast cancer.

A number of Lx compounds were tested for anti 5-LOX and 12-LOX activities in the human breast cancer cell line, MCF-7. The results in Fig 6 show that the Lx compounds exhibit inhibitory effects against 5-LOX and 12-LOX activities. The ability of 5-LOX and 12-LOX to produce oxidated products of 20:4n-6 with a different

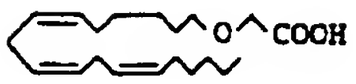
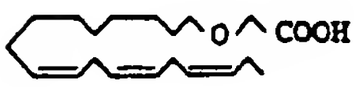
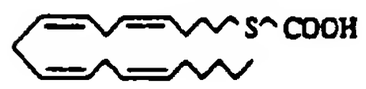
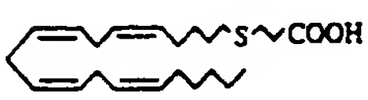
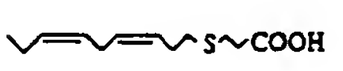
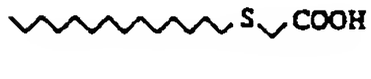
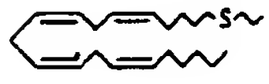
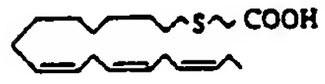
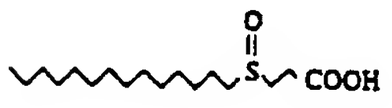
absorption wavelength in the presence of nitroso-PUFA was examined and is shown in the progress curves in Fig 6. Both Lx7 and Lx9 can activate 5-LOX (Figs 6A and 6B) in a purified enzyme system. Within cells, activation of 5-LOX is facilitated by 5-LOX activating protein (FLAP). Thus, in intact cells, Lx7 and Lx9 may be able to activate 5-LOX at lower concentrations than those required in the purified enzyme system shown in Fig 6. Lx7 is also a substrate for 12-LOX (Fig 6C) and may compete with 20:4n-6 *in vivo* resulting in the loss of 12-LOX growth promoting products whereas Lx9 is a direct, potent and rapid inhibitor of 12-LOX (Fig 6D). By being able to activate 5-LOX and suppress 12-LOX, these compounds have the desired characteristics of anti-breast cancer agents as discussed previously.

While interference with 5-LOX or 12-LOX activity individually impacts on growth rates of human breast cancer MCF-7 cells[55,56], the simultaneous modification of the activities of both enzymes at once may cause death. When Lx7 and Lx9 were added to MCF-7 cells *in vitro*, these two compounds were found to kill these tumour cells (Fig 7) at concentrations below those at which 20:4n-6 became toxic. Lx4, a nitroso-compound with the same number of carbon atoms and degree of unsaturation as Lx9 but lacking a COOH group, was ineffective (Fig 7).

D. PROPERTIES OF THE β AND γ OXA AND THIA FATTY ACIDS

Other analogues of PUFAs targeted in this project were the oxa and thia fatty acids, owing to their potential as antioxidants, and therefore corresponding potential as anti-cancer agents. Compounds of types **16-19**, as identified in Table 4, were constructed as PUFA analogues having the property of resistance to β-oxidation [67,13].

Table 4. **Structure and nomenclature of the oxa and thia fatty acid analogues and other thia compounds**

Structure	Systematic name	WCH	Thesis
	(Z,Z,Z)-(octadeca-6,9,12-trienyloxy) acetic acid	16	MP4
	(Z,Z,Z)-(octadeca-9,12,15-trienyloxy) acetic acid	17	MP5
	(all-Z)-(eicosa-5,8,11,14-tetraenylthio) acetic acid	18	MP8
	3-[(all-Z)-(eicosa-5,8,11,14-tetraenylthio) propionic acid	19	MP11
	3-[(3Z,6Z)-nona-3,6-dienylthiopropionic acid	106	
	3-tetradecylthiopropionic acid	108	
	2-tetradecylthiopropionic acid	109	
	propyl(all-Z)-eicosa-5,8,11,14-tetraenylpropyl sulfide	110	
	propyltetradecyl sulfide	111	
	3-[(Z,Z,Z)-(octadeca-9,12,15-trienylthio)]propionic acid	112	MP13
	3-(tetradecylsulfinyl) propionic acid	113	
	2-(tetradecylsulfinyl) acetic acid	114	

Experimental

^{1}H NMR and ^{13}C NMR spectra were recorded on a Gemini 300 MHz or a Unity Inova 500 MHz spectrometers with tetramethylsilane (TMS) as the internal standard (δ 0.00 ppm). Samples were run in deuterochloroform (99.8% D) unless indicated

otherwise. The following abbreviations are adopted: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublets); bs (broad singlet). *J* values are given in Hz.

Infrared (IR) spectra were recorded on Perkin-Elmer 683 and 7700 infrared spectrophotometers. The following abbreviations are used: br (broad), w (weak), m (medium), s (strong).

Ultraviolet spectra were recorded on a Shimadzu UV 2101 PC spectrophotometer with a temperature controller and kinetic software.

Low and high resolution electron ionisation (EI) mass spectra and chemical ionisation (CI) mass spectra were run on a Fisons VG Autospec. A Fisons VG Instrument Quattro II mass spectrometer was used for negative ion electrospray mass spectra. Gas chromatography-mass spectrometry (GC-MS) was carried out with a HP 5970 mass selective detector connected to a HP 5890 gas chromatography with a 12.5 m BP-1 column.

Melting points were determined using a Reichert microscope with a Köfler heating stage and are uncorrected. Buffers were adjusted to the required Ph using a model 520A pH meter. Microanalyses were conducted by the Microanalytical Laboratory, Research School of Chemistry, Australian National University.

HPLC was performed using a Waters HPLC system with ultraviolet (UV) or refractive index (RI) detection. The column used contained Alltech Spherisorb octadecylsilane (ODS) (4.6 mm x 250 mm, 3 μm). The mobile phase was comprised of acetonitrile (or methanol) and phosphoric acid (30 mM) solution in the ratios indicated in the text, with a flow rate of 1 ml/min.

Column chromatography was carried out using Merck Silicagel 60 as the absorbent. Analytical TLC was performed on Merck Silicagel 60 F_{254} silica on aluminium baked plates.

15-LO was obtained from Sigma Chemical Company, and 12-LO from Cayman Chemical Company. Arachidonic acid 1, linolenyl alcohol 57a, gamma linolenyl alcohol 57b, arachidonyl alcohol 57c and docosahexaenyl alcohol 57d were purchased from Nu-Chek Prep. Inc. Elysian, Minnesota, USA. Other chemicals were commercially available from Aldrich Chemical Company.

Determination of stability of thia fatty acids and sulfides

Compounds 110 (4.3 mg) and 106 (6 mg) were each dissolved in 5 ml of dichloromethane and added into 250 ml one-neck flasks. Compound 18 (20 mg) and compounds 19, 108, 109 and 111-112 (14-20 mg) were each dissolved in 10 ml of dichloromethane and added into 500 ml flasks. The solvent dichloromethane was then evaporated with continuous rotation of the flasks, allowing the compounds to form thin films. The flasks were flushed with oxygen, sealed and kept in darkness. The compounds in the flasks were redissolved in chloroform-*d* and analysed by ^1H NMR every two weeks for up to six weeks.

Determination of antioxidant behaviour of 3-[(3Z,6Z)-nona-3,6-dienylthio]propionic acid on arachidonic acid autoxidation

This is a typical autoxidation assay designed to investigate the antioxidant properties of thia fatty acids and sulfides in the autoxidation of arachidonic acid 1.

A stock solution in dichloromethane (2 ml) containing arachidonic acid 1 (18 mg) and 3-[(3Z,6Z)-nona-3,6-dienylthio]propionic acid 106 (18 mg) was prepared with lauric acid (18 mg) as an internal standard. Samples of the stock solution (100 µl) were added to glass Petri-dishes followed by ethanol (400 µl). After evaporation of the solvent, a well-distributed thin film was formed on each Petri-dish. The Petri-dishes were placed in a desiccator, which was then evacuated, filled with oxygen and stored in the darkness. Dishes were removed from the desiccator after 1, 2, 3, 5 and 7 days. The mixture on each dish was redissolved in diethyl ether and transferred to a 2 ml vial. After evaporation of the solvent, the residue was dissolved in the HPLC mobile phase (100 µl) and 10% of the solution was analysed by HPLC using a reverse phase column (octadecylsilane) (4.6 mm x 250 mm, 3 µm) and a refractive index detector. Table 5 shows the mobile phases used for different thia fatty acids and sulfides, and their retention times by HPLC.

Table 5 HPLC mobile phase and retention time of thia fatty acids and sulfides

Compound	Mobile phase (Buffer = 30 mM H_3PO_4)	Retention time (min) (Arachidonic acid 1)	Retention time (min) (Lauric acid)	Retention time (min) (Compound)
18	Acetonitrile-Buffer (80 : 20)	6.53	4.23	8.75
19	Acetonitrile-Buffer (80 : 20)	6.80	4.44	10.91
106	Acetonitrile-Buffer (70 : 30)	14.71	7.13	3.15
108	Methanol-Buffer (90 : 10)	6.71	4.00	10.74
109	Methanol-Buffer (90 : 10)	6.82	4.05	9.38
110	Acetonitrile-Buffer (95 : 5)	3.48	3.09	14.05
111	Acetonitrile-Buffer (95 : 5)	3.38	3.05	21.57
112	Acetonitrile-Buffer (90 : 10)	5.24	3.80	6.97

Synthesis of analogues of 3-[(*all-Z*)-(eicosa-5,8,11,14-tetraenyl-thio)]propionic acid

Pent-2-ynyl *p*-toluenesulfonate, 102. 2-Pentyn-1-ol 101 (1.03 g, 12 mmol) was dissolved in chloroform (10 ml) and the mixture was cooled in an ice bath. Pyridine (1.90 g, 24 mmol, 2 eq) was then added, followed by *p*-toluenesulfonyl chloride (3.43 g, 18 mmol, 1.5 eq) in small portions with constant stirring. The reaction was complete in 4 h (monitored by TLC). Ether (30 ml) and water (7 ml) were added and the organic layer was washed successively with 1 N HCl (7 ml), 5% $NaHCO_3$, water (7 ml) and brine (20 ml), and then dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude tosylate was flash column chromatographed on silica gel using ether-hexane (20 : 80) as the eluent to yield the title compound 102 (1.85 g, 65%) as a colourless oil. Found: C, 60.24; H, 5.93; S, 13.22. Calc. for $C_{12}H_{14}SO_3$: C, 60.48; H, 5.92; S, 13.45%. ν_{max} (film)/cm^{-1} 2980 (m), 2940 (w), 2878 (w), 2240 (m), 1598 (s), 1495 (w), 1450 (m), 1360 (s), 1180 (s), 1175 (s), 1095 (s), 1020 (m), 1000 (m), 960 (s), 940 (s), 840 (s), 815 (s), 735 (s), 662 (s); δ_H (300 MHz, $CDCl_3$) 0.98-1.03 (3H, m, C5-H_3); 2.04-2.10 (2H, m, C4-H_2), 2.44 (3H, s, ArCH_3), 4.69 (2H, m, C1-H_2), 7.35 and 7.82 (4H, dd, *J* 8.3 and 8.7, ArH); δ_C (300 MHz, $CDCl_3$) 12.91, 13.72, 22.22, 59.35, 71.72, 92.33, 128.69, 130.30, 133.90, 145.47; *m/e* (EI): 238 (M^+, <0.1%), 209 (1), 155 (24), 139 (100), 129 (6), 117 (18), 107 (10), 92 (42), 91 (87), 83 (29), 66 (50), 65 (48).

Nona-3,6-diyn-1-ol, 103. Pent-2-ynyl *p*-toluenesolfonate 102 (1.37 g, 5.78 mmol, 1.1 eq) was added at -30 °C under nitrogen to a well-stirred suspension in DMF (15ml) of but-3-yn-1-ol (368 mg, 5.25 mmol, 1 eq), sodium carbonate (834 mg, 7.87 mmol, 1.5 eq), tetrabutylammomium chloride (1.46 g, 5.25 mmol) and copper(I) iodide (1.00 g, 5.25 mmol, 1 eq). The mixture was stirred at room temperature for 48 h. Ether (30 ml) and 1M HCl (30 ml) were then added. After filtration through a bed of celite, the organic phase was washed with brine, dried over sodium sulfate and the solvent was evaporated under reduced pressure. Purification of the residue by flash column chromatography on silica gel with ether-hexane (40 : 60) as the eluent gave the

product 103 (442 mg, 62%) as a colourless oil. Found: C, 79.55; H, 8.82. Calc. for $C_9H_{12}O$: C, 79.37; H, 8.88%. ν_{max} (film)/cm^{-1} 3650-3100 (br), 2975 (s), 2938 (s), 2905 (s), 2880 (s), 2500 (m), 1415 (m), 1375 (w), 1320 (s), 1180 (w), 1120 (w), 1040 (s), 900 (m), 735 (w); δ_H (300 MHz, $CDCl_3$) 1.10 (3H, t, *J* 7.4, C9-H_3), 1.96 (H, bs, OH), 2.13-2.20 (2H, m, C8-H_2), 2.41-2.45 (2H, m, C2-H_2), 3.11-3.13 (2H, m, C5-H_2), 3.69 (2H, t, *J* 6.1, C1-H_2); δ_C (Acetone, 300 MHz) 10.14, 13.07, 14.72, 24.03, 61.95, 75.08, 76.83, 78.46, 82.42; *m/e* (EI): 135 [$(M-H)^+$, 12%], 121 (44), 107 (30), 105 (51), 103 (29), 93 (44), 91 (100), 79 (58), 77 (80), 65 (41), 63 (29), 57 (14), 53 (27), 51 (37); HRMS: found *m/e* 135.081144 $(M-H)^+$; calc. for $C_9H_{11}O$: 135.080990.

(3Z,6Z)-Nona-3,6-dien-1-ol, 104. Nona-3,6-diyn-1-ol 103 (198 mg, 1.45 mmol) was hydrogenated at atmospheric pressure, in the presence of a mixture of quinoline (44 mg) and palladium (5%) on calcium carbonate (100 mg), poisoned with lead in methanol (25 ml). The reaction was stopped after 2.5 h when the uptake of hydrogen was 61 ml. Removal of methanol *in vacuo*, followed by silica gel column chromatography to remove quinoline using ether-hexane (35 : 65) as the eluent gave 187 mg (92%) of (3Z, 6Z)-nona-3,6-dien-1-ol 104 as a colourless oil. Found: C, 77.42; H, 11.75. Calc. for $C_9H_{16}O$: C, 77.09; H, 11.50%. ν_{max} (film)/cm^{-1} 3500-3160 (br), 3011 (s), 2960 (s), 2930 (s), 2870 (s), 1462 (m), 1377 (m), 1050 (m), 722 (m); δ_H (300 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.6, H9-H_3), 2.01-2.12 (2H, m, C8-H), 2.32-2.40 (2H, m, C2-H_2), 2.79-2.84 (2H, t, *J* 7.1, C5-H_2), 3.64 (2H, m, C1-H_2), 5.27-5.43 (3H, m), 5.49-5.56 (H, m); δ_C (300 MHz, $CDCl_3$) 14.82, 21.14, 26.20, 31.33, 62.77, 125.90, 127.40, 132.04, 132.74; *m/e* (EI): 140 (M^+, 2%); 122 (15), 111 (7), 109 (12), 107 (22), 98 (12), 96 (19), 95 (21), 93 (72), 91 (33), 81 (39), 79 (56), 68 (31), 67 (100), 55 (59), 54 (21), 53 (21); HRMS: found *m/e* 140.120290 (M^+); calc. for $C_9H_{16}O$: 140.120115.

(3Z,6Z)-Nona-3,6-dienyl *p*-toluenesulfonate, 105. (3Z,6Z)-Nona-3,6-dien-1-ol 104 (167 mg, 1.19 mmol) was dissolved in chloroform (5 ml) and the solution was cooled in an ice bath. Pyridine (376 mg, 4.76 mmol, 4 eq) was then added, followed by the addition of *p*-toluenesulfonyl chloride (340 mg, 1.78 mmol, 1.5 eq) in small portions

with constant stirring. The mixture was stirred for 24 h at 15 °C. Ether (15 ml) and water (5 ml) were added and the organic layer was washed successively with 1 N HCl (10 ml), 5% $NaHCO_3$, water (10 ml), and brine (10 ml), and then dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude tosylate was flash column chromatographed on silica gel with ether-hexane (20 : 80) as the eluent to yield starting material (15 mg, 9%) and the title product 105 (201 mg, 57%) as a colourless oil. Found: C, 65.17; H, 7.44; S, 11.27. Calc. for $C_{16}H_{22}SO_3$: C, 65.28; H, 7.53; S, 10.89%. ν_{max} (film)/cm^{-1} 3005 (m), 2960 (s), 2930 (m), 2870 (m), 1599 (m), 1462 (m), 1377 (s), 1310 (w), 1290 (w), 1189 (s), 1178 (s), 1100 (s), 1020 (w), 973 (s), 815 (s), 770 (m), 660 (s); δ_H (300 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.6, C9-H_3), 2.00-2.05 (2H, m, C8-H_2), 2.38-2.44 (2H, m, C2-H_2), 2.45 (3H, s, ArCH_3), 2.69-2.74 (2H, t, *J* 7.0, C5-H_2), 3.99-4.04 (2H, m, C1-H_2), 5.20-5.28 (2H, m), 5.34-5.50 (2H, m) 7.33, 7.80 (4H, dd, *J* 8.2 and 8.7, AA'BB' and ArH); δ_C (300 MHz, $CDCl_3$) 14.78, 21.09, 22.20, 26.12, 27.64, 70.20, 123.53, 126.94, 128.47, 130.37, 132.61, 132.92, 145.28; *m/e* (EI): [277 $(M\text{-}OH)^+$, 1%], 155 (25), 139 (2), 122 (67), 107 (47), 93 (100), 91 (77), 79 (66), 67 (47), 55 (32); *m/e* (CI): 312 $(M+NH_4)^+$.

3-[(3Z,6Z)-Nona-3,6-dienylthio]propionic acid, 106. 3-Mercaptopropionic acid (150 mg, 1.41 mmol, 1.5 eq) was added, under an atmosphere of dry nitrogen, to a stirred solution of sodium methoxide, prepared from sodium (64 mg, 2.78 mmol, 3 eq) and methanol (20 ml). After the initial white precipitate had dissolved, a solution of (3Z,6Z)-nona-3,6-dienyl *p*-toluenesulfonate 105 (276 mg, 0.94 mmol) in diethyl ether was added. The mixture was stirred at 40 °C for 2 days under nitrogen, then hydrochloric acid (10% v/v, 20 ml) and diethyl ether (20 ml) were poured into the crude reaction mixture. The organic phase was separated and washed with water and brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by flash column chromatography using ether-hexane-acetic acid (60 : 40 : 2) as the eluent to afford 3-[(3Z,6Z)-noca-3,6-dienylthio]propionic acid 106 (88 mg, 41%) as a colourless oil. Found: C, 62.90; H, 8.73; S, 14.01. Calc. for $C_{12}H_{20}SO_2$: C, 63.12; H, 8.83; S, 14.04%. ν_{max} (film)/cm^{-1} 3400-2500 (br), 3005 (m), 2960 (m), 2910 (m), 2870

(w), 1713 (s), 1459 (m), 1377 (w), 1264 (m), 1195 (w), 1140 (w), 940 (w); δ_H (500 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.8, C9'-H_3), 2.05-2.08 (2H, m, C8'-H_2), 2.34-2.39 (2H, m, C2'-H_2), 2.57-2.60 (2H, t, *J* 7.4, C1'-H_2), 2.65-2.69 (2H, t, *J* 7.3, C3-H_2), 2.78-2.82 (4H, m, C5'-H_2, C2-H_2), 5.27-5.32 (H, m), 5.37-5.47 (3H, m), 5.50-6.10 (H, bs, COOH); δ_C (300 MHz, $CDCl_3$) 14.83, 21.14, 26.20, 27.19, 27.95, 32.62, 35.21, 127.37, 127.97, 130.53, 132.72, 178.66; *m/e* (EI): 228 (M^+, 34%), 169 (14), 159 (18), 155 (45), 133 (8), 122 (54), 119 (42), 113 (12), 107 (44), 93 (100), 89 (66), 79 (57), 77 (53), 67 (52), 61 (33), 55 (43); HRMS: found *m/e* 228.118179 (M^+); calc. for $C_{12}H_{20}SO_2$: 228.118402.

3-Tetradecylthiopropionic acid, 108. According to the procedure described for the preparation of 3-[3Z,6Z)-nona-3,6-dienylthio]propionic acid 106, 3-mercaptopropionic acid (261 mg, 2.46 mmol, 1.2 eq) was added, under an atmosphere of dry nitrogen, to a stirred solution of sodium methoxide prepared from sodium (142 mg, 6.17 mmol, 3 eq) and methanol (20 ml). After the initial white precipitate had dissolved, a solution of 1-bromotetradecane 107 (568 mg, 2.05 mmol) in diethyl ether (2 ml) was added. The reaction mixture was stirred for 16 h at room temperature. After workup and purification by flash column chromatography using ether-hexane (20 : 80) → ether-hexane-acetic acid (60 : 40 : 1) for elution, the title compound 108 (450 mg, 73%) was obtained as a white solid, mp: 67 °C. Found: C, 67.32; H, 11.32; S, 10.41. Calc. for $C_{17}H_{34}SO_2$: C, 67.50; H, 11.33; S, 10.60%. ν_{max} (Nujol)/cm^{-1} 3100-2600 (br), 2965 (s), 2910 (s), 2840 (s), 1680 (s), 1460 (s), 1405 (w), 1375 (m), 1265 (m), 1255 (w), 1231 (w), 1210 (w), 1200 (m), 1080 (w), 915 (m), 725 (m); $\square_H$ (500 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.7, C14'-H_3), 1.25-1.38 [22H, m, (C3'-C13')-H_2], 1.56-1.61 (2H, m, C2'-H_2), 2.54 (2H, bs, C1'-H_2), 2.65-2.68 (2H, t, *J* 6.6, C3-H_2), 2.79 (2H, bs, C2-H_2); δ_C (300 MHz, $CDCl_3$) 14.69, 23.26, 27.16, 29.44, 29.80, 29.93, 30.02, 30.10, 30.17, 30.23, 32.49, 32.78, 35.25, 178.50; *m/e* (EI): 302 (M^+, 21%), 230 (24), 229 (100), 185 (2), 161 (4), 119 (8), 106 (24), 97 (15), 89 (21), 83 (22), 69 (25), 55 (32); HRMS: found *m/e* 302.227166 (M^+); calc. for $C_{17}H_{34}SO_2$: 302.227952.

2-Tetradecylthioacetic acid, 109. 2-Mercaptoacetic acid (288 mg, 3.13 mmol, 1.2 eq) was added, under an atmosphere of dry nitrogen, to a stirred solution of sodium methoxide, prepared from sodium (180 mg, 7.83 mmol, 3 eq) and methanol (20 ml). After the initial white precipitate had dissolved, a solution of 1-bromotetradecane 107 (725 mg, 2.61 mmol) in diethyl ether (2 ml) was added and the mixture was stirred for 16 h at room temperature under nitrogen. The crude reaction mixture was poured into an equal volume of hydrochloric acid (10% v/v), and the organic phase was separated and washed with water and brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by flash column chromatography using diethyl ether-hexane (20 : 80) → diethyl ether-hexane-acetic acid (60 : 40 : 2) for elution and crystallised to afford 2-tetradecylthioacetic acid 109 (580 mg, 77%) as a white solid, mp: 68 °C. Found: C, 66.46; H, 10.93; S, 10.83. Calc. for $C_{16}H_{32}SO_2$; C, 66.61; H, 11.18; S, 11.11%. ν_{max} (Nujol)/cm^{-1} 3200-2600 (br), 2950 (s), 2910 (s), 2840 (s), 1680 (s), 1460 (s), 1425 (w), 1375 (s), 1265 (m), 1140 (w), 908 (w), 725 (w); δ_H (300 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.6, C14'-H_3), 1.26-1.40 [22H, m, (C3'-C13')-H_2], 1.56-1.64 (2H, m, C2'-H_2), 2.64-2.69 (2H, t, *J* 7.4, C1'-H_2), 3.26 (2H, s, C2-H_2); δ_C (300 MHz, $CDCl_3$) 14.68, 23.26, 29.30, 29.46, 29.75, 29.93, 30.06, 30.15, 30.22, 32.49, 33.36, 34.05, 177.57; *m/e* (EI): 288 (M^+, 12%), 230 (21), 229 (100), 111(6), 97 (17), 83 (27), 69 (30), 55 (34); HRMS: found *m/e* 288.212125 (M^+); calc. for $C_{16}H_{32}SO_2$: 288.212302.

Propyl (*all*-*Z*)-eicosa-5,8,11,14-tetraenyl sulfide 110. Using the procedure described for the preparation of 3-tetradecylthiopropionic acid 108, propanethiol (26 mg, 0.34 mmol, 1.2 eq) was added, under an atmosphere of dry nitrogen, to a stirred solution of sodium methoxide, prepared from sodium (20 mg, 0.87 mmol, 3 eq) and methanol (10 ml). After the initial white precipitate had dissolved, a solution of (*all*-*Z*)-1-bromo-5,8,11,14-eicosatetrane 58c (101 mg, 0.29 mmol) in diethyl ether (1 ml) was added. The reaction mixture was stirred for 15 h at room temperature. After workup, purification by flash column chromatography using hexane for elution gave the title compound 110 (75 mg, 75%) as a colourless oil. Found: C, 78.91; H, 11.38; S, 8.96. Calc. for $C_{23}H_{40}S$: C, 79.24; H, 11.56; S, 9.20%. ν_{max} (film)/cm^{-1} 3005 (s), 2950 (s), 2920 (s),

2850 (s), 1650 (w), 1450 (m), 1390 (w), 1375 (w), 1290 (w), 1260 (w), 1230 (w), 910 (w), 720 (m); δ_H ($CDCl_3$, 300 MHz) 0.89 (3H, t, *J* 6.8, C20-H_3), 0.99 (3H, t, *J* 7.2, C3'-H_3), 1.26-1.35 (6H, m, C17-H_2, C18-H_2, C19-H2), 1.43-1.48 (2H, C3-H2), 1.57-1.64 ($_4$H, m, C2-H2, C2'-H2), 2.05-2.13 ($_4$H, m, C_4-H2, C16-H2), 2.50-2.51 (4H, m, C1-H2, C1'-H2), 2.80-2.86 (6H, m, C7-H2, C10-H2, C13-H2), 5.32-5.43 (8H, m, C5-H, C6-H, C8-H, C9-H, C11-H, C12-H, C14-H, C15-H); δ_C ($CDCl_3$, 300 MHz) 14.13, 14.67, 23.17, 23.60, 26.22, 27.41, 27.81, 29.44, 29.91, 32.11, 32.54, 34.79, 128.12, 128.48, 128.64(2C), 128.90, 129.11, 130.40, 131.06; *m/e* (EI): 348 (M^+, 44%), 305 (38), 273 (4), 251 (6), 237 (14), 205 (17), 177 (19), 161 (36), 150 (27), 131 (29), 119 (40), 105 (48), 93 (77), 91 (76), 81 (79), 79 (95), 67 (100), 55 (69); HRMS: found *m/e* 348.285378 (M^+); calc. for $C_{23}H_{40}S$: 348.285073.

Propyl tetradecyl sulfide, 111. Using the procedure described above for the synthesis of propyl (*all*-*Z*)-eicosa-5,8,11,14-tetraenyl sulfide 110, propanethiol (165 mg, 2.16 mmol, 1.2 eq) was added, under an atmosphere of dry nitrogen, to a stirred solution of sodium methoxide, prepared from sodium (82 mg, 3.56 mmol, 2 eq) and methanol (10 ml). After the initial white precipitate had dissolved, a solution of 1-bromotetradecane 107 (500 mg, 1.80 mmol) in diethyl ether (2 ml) was added. The reaction mixture was stirred for 15 h at room temperature. After workup, purification by flash column chromatography using hexane for elution gave the title compound 111 (435 mg, 89%) as a colourless oil. Found: C, 75.05; H, 13.27; S, 11.50. Calc. for $C_{17}H_{36}S$: C, 74.92; H, 13.31; S, 11.76%. ν_{max} (film)/cm^{-1} 2960 (s), 2910 (s), 2850 (s), 1460 (s), 1375 (w), 1290 (w), 1270 (w), 890 (w), 720 (w); δ_H ($CDCl_3$, 300 MHz) 0.87 (3H, t, *J* 6.5, C14-H_3), 0.99 (3H, t, *J* 7.4, C3'-H_3), 1.25 [22H, m, (C3-C13)-H_2], 1.54-1.63 (4H, m, C2-H_2, C2'-H_2), 2.47-2.51 (4H, m, C1-H_2, C1'-H_2); δ_C ($CDCl_3$, 300 MHz) 14.13, 14.71, 23.28, 23.59, 29.55, 29.85, 29.94, 30.12, 30.18, 30.23, 30.33, 32.50, 32.69, 34.78; *m/e* (EI): 272 (M^+, 52%), 243 (18), 229 (100), 196 (8), 187 (2), 168 (5), 145 (6), 131 (15), 111 (14), 97 (22), 89 (34), 83 (27), 76 (33), 69 (32), 57 (30), 55 (44).

3-(Tetradecylsulfinyl)propionic acid, 113. Arachidonic acid 1 (175 mg) was dissolved in 5 ml of dichloromethane to make a stock solution (35 mg/ml). 3-Tetradecylthiopropanoic acid 108 (10 mg, 0.03 mmol), arachidonic acid 1 (10 mg, 0.03 mmol, 284 µl) and dichloromethane (10 ml) were added into a one-neck flask (500 ml). The solvent was evaporated using a rotary evaporator to allow the reagents to form a thin film on the internal surface of the flask. The flask was filled with oxygen and placed in darkness for 7 days. Dichloromethane (5 ml) was then added into the flask to dissolve the mixture and the solution was then transferred to a 2 ml vial. After evaporation of the solvent, the residue was dissolved in 300 µl of the mobile phase (methanol-30 mM phosphoric acid, 90 : 10) and then subject to reverse phase HPLC analysis. The HPLC was performed on an Alltech Spherisorb octadecylsilane (ODS) column with RI detection. The flow rate of the mobile phase was 3 ml/min. Fifty microlitres of the sample was loaded each time. The product with a retention time of 5.49 min was collected and pooled. After evaporation of the solvent at reduced pressure, the product was extracted with diethyl ether (2 ml). The resulting extract was washed with water and dried with Na_2SO_4 and the solvent evaporated, yielding the title compound 113 (2 mg) as a white solid, mp: 166-167 °C. Found: 64.33, H, 10.50. Calc. for $C_{17}H_{34}SO_3$: C, 64.11; H, 10.76%. ν_{max} (Nujol)/cm^{-1} 3600-2500 (br), 2965 (s), 2910 (s), 2840 (s), 1695 (m), 1460 (s), 1375 (s), 1330 (w), 1305 (w), 1125 (w), 1040 (w), 1025 (w), 920 (w), 720 (w); δ_H ($CDCl_3$, 500 MHz) 0.81 (3H, t, *J* 7.0, C14'-H_3), 1.19-1.26 [20H, m, C4'-C13')-H_2], 1.34-1.37 (2H, m, C3'-H_2), 1.68-1.72 (2H, m, C2'-H_2), 2.70-2.76 (H, m), 2.82-2.89 (3H, m), 2.88-3.03 (H, m), 3.05-3.10 (H, m), 7.96 (H, bs, COOH); δ_C ($CDCl_3$, 300 MHz) 14.67, 23.19, 23.24, 27.78, 29.29, 29.72, 29.91, 30.09, 30.17, 30.20, 32.47, 46.66, 52.53, 174.37; *m/e* (CI): 319 (MH^+); *m/e* (EI): 301 [$(M\text{-}OH)^+$, 27%], 246 (21), 245 (16), 229 (100), 196 (5), 121 (15), 94 (22), 97 (22), 83 (29), 71 (32), 70 (34), 57 (51); HRMS: found *m/e* 301.219714 $(M\text{-}OH)^+$; calc. for $C_{17}H_{33}SO_2$: 301.220127.

2-(Tetradecylsulfinyl)acetic acid, 114. 2-Tetradecylthioacetic acid 109 (19mg, 0.066 mmol) was dissolved in dichloromethane (2 ml) and *tert*-butylhydroperoxide (11 ml, 0.08 mmol, 1.2 eq) was added. After 48 h reaction at room temperature, the solvent was removed and the residue was chromatographed using ether-hexane-acetic acid (60 : 40 : 2) → methanol as the eluent to obtain the white product 114 (17 mg, 86%). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, *J* 6.4, C14'-H_3), 1.20-1.29 [20H, m, (C4'-C13)-H_2], 1,44-1.50 (2H, m, C3'-H_2), 1.77-1.82 (2H, m, C2'-H_2), 2.88-2.95 (H, m, C1'-H), 3.02-3.07 (H, m, C1'-H'), 3.63-3.68 (H, d, *J* 14, C2-H), 3.81-3.86 (H, d, *J* 14, C2-H'), 7.92 (H, bs, COOH); δ_C ($CDCl_3$, 300MHz) 14.69, 23.20, 23.26, 29.18, 29.70, 29.89, 29.93, 30.09, 30.18, 30.22, 32.49, 52.27, 53.47, 166.93; *m/e* (EI): 305 [$(M+1)^+$, 1%], 287 (50), 243 (60), 229 (94), 196 (12), 168 (6), 149 (6), 125 (10), 111 (21), 97 (45), 83 (63), 69 (74), 57 (100), 55 (91); HRMS: found *m/e* 305.215275 $(M+1)^+$ calc. for $C_{16}H_{33}SO_3$: 305.215042.

CONCLUSION

The main group of compounds targeted in this project was the nitro analogues of PUFAs. They were expected to be potentially useful due to their generally high stability and the chemical similarity of the nitro group to the carboxyl group.

From the nine nitro analogues of PUFAs that were synthesised, including long chain nitroalkanes, γ-nitro fatty acids and carboxyethyl nitro fatty acids, (all-Z)-4-nitrotricosa-8,11,14,17-tetraenoic acid has been identified as a good substrate of soybean 15-LO and a 12-LO from porcine leukocytes. The substrate activity of this compound with the soybean 15-LO is comparable to that of arachidonic acid, which is a major substrate of the lipoxygenase.

A more significant outcome of this work was the identification of 4-nitrohenicosanoic acid, 3-(all-Z)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic acid and 3-heptadecyl-3-nitropentane-1,5-dicarboxylic acid as selective inhibitors of 5-LO, 12-LO and 15-LO catalysed oxidation of arachidonic acid, respectively. Although a large number of inhibitors have been reported for these three lipoxygenases, so far few inhibitors have entered clinical trials and no agents that are selective for 15-LO *vs* 5-LO (or *vs* 12-LO) are available. [65]

Selective inhibition of a specific lipoxygenase is particularly desirable for treatment of diseases related to these metabolic pathways. Non-selective inhibitors have the disadvantages of causing possible side effects. For instance, asthma has been treated as an inflammatory disease, and corticosteroids are the therapy of choice for the inflammatory component of asthma. [66] Although this class of drugs provides powerful anti-inflammatory effects in most patients, these effects are not specific and in some cases result in serious side effects. Since leukotrienes, a family of inflammatory mediators generated through the 5-LO pathway, have been shown to enhance bronchoconstriction and airway mucus secretion, agents that target the specific inflammatory pathway have been developed to treat asthma by modulating leukotriene activity. So far, specific leukotriene receptor antagonists and synthesis inhibitors have been extensively studied in laboratory-induced asthma and currently show promise in clinical trials; one leukotriene receptor antagonist (zafirlukast) and one 5-LO inhibitor (zileuton) were recently approved for the treatment of asthma. [66] The identification of the three nitro analogues of PUFAs having selective inhibition activity with the three lipoxygenases may lead toward a new class of drugs with specificity and reduced side effects for treating diseases that are associated with lipoxygenase pathways.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

REFERENCES

1. Ferrante, A., Hii, C.S.T., Huang, Z.H., Rathjen, D.A. In *The Neutrophils: New Outlook for the Old Cells.* (Ed. Gabrilovich, D.) Imperial College Press (1999) 4: 79-150.

2. Sinclair, A., and Gibson, R. (eds) 1992. Invited papers from the Third International Congress. American Oil Chemists' Society, Champaign, Illinois 1-482.

3. Abel, S., Gelderblom, W.C.A., Smuts, C.M., Kruger M. *Pros. Leuko. and Essential,* 56 (1): 29-39 (1997).

4. Krombout, D. *Nutr. Rev.* 50:49-53 (1992).

5. Kinsella, J.E., Lokesh, B., Stone R.A. *Am. J. Clin. Nutr.* 52:1-28 (1990)

6. Kumaratilake, L.M., Robinson, B.S., Ferrante, A., Poulos A. *J. Am. Soc. Clin. Investigation* 89: 961-967 (1992).

7. Weber, P.C. *Biochem. Soc. Trans.* 18: 1045-1049 (1990).

8. Arm, J.P., and Lee, T.H. *Clin. Sci.* 84: 501-510 (1993).

9. Thien, F.K.C.K., and Walters, E.H. *Pros. Leuko and Essential* 52: 271-288 (1995).

10. Ford-Hutchinson, A.W. *Crit. Rev. Immunol.* 10(1) : 1 (1990).

11. Bates, E.J. *Pros. Leuko and Essential.* 53: 75-86 (1995).

12. Ferrante, A., Poulos, A., Easton, C.J., Pitt, M.J., Robertson, T.A., Rathjen, D.A. International Patent Application No. PCT/AU95/00677 (1995)-WO96/11908: *Chem. Abstr.* 125: 58194 (1996).

13. Pitt, M.J., Easton, C.J., Moody, C.J., Ferrante, A., Poulos, A., and Rathjen, D.A. *Synthesis* 11:1239-1242 (1997).

14. Barnes, N.C., Hui, P.K. *Pulmonary Pharmacol.* 6(1): 3-9 (1993).

15. Kornblum, N., Taub, B., Ungnade, H.E. *J. Am. Chem. Soc.* 76: 3209-3211 (1954).

16. Chasar, D.W. *Synthesis* 841-842 (1982).

17. Pollini, G.P., Barco, A., and de Guili, G. *Synthesis.* 44-45 (1972).

18. Corey, E.J., and Suggs, J.W. *Tetrahedron Letters* 31:2647-2650 (1975).

19. Rosini, G., Ballini, R., and Petrini, M. *Synthesis* 269-271 (1985).

20. Melton, J., and McMurry, J.E. *J. Org. Chem.* 4(14) (1975).

21. Finkbeiner, H.L., and Wagner, G.W. *J. Org. Chem.* 28: 215 (1963).

22. Finkbeiner, H.L., and Stiles, M. *J. Am. Chem. Soc.* 85: 616-632 (1962).

23. Hayashi, H.; Nakanishi, K.; Brandon, C.; Marmur, J. *J. Am. Chem. Soc.* **1973**, *95*, 8749.

24. Kornblum, N.; Taub, B.; Ungnade, H. E. *J. Am. Chem. Soc.* **1954**, *76*, 3209.

25. a) Stiles, M.; Finkbeiner, H. L. *J. Am. Chem. Soc.* **1959**, *81*, 505.

b) Finkbeiner, H. L.; Wagner, G. W. *J. Org. Chem.* **1963**, *28*, 215.

26. Seebach, D.; Lehr, F. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 505.

27. a) Finkbeiner, H. L.; Stiles, M. *J. Am. Chem. Soc.* **1963**, *85*, 616.

b) Feuer, H.; Hass, H. B.; Warren, K. S. *J. Am. Chem. Soc.* **1949**, *71*, 3078.

28. Chasar, D. W. *Synthesis* **1982**, 841.

29. Baldwin, J. E.; Au, A.; Christie, M.; Haber, S. B.; Hesson, D. *J. Am. Chem. Soc.* **1975**, *97*, 5957.

30. Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *31*, 2647.

31. Rosini, G.; Ballini, R. *Synthesis* **1988**, 833.

32. Ballini, R.; Bosica, G.; Forconi. P. *Tetrahedron* **1996**, 52, 1677.

33. Melton, J.; McMurry, J. E. *J. Org. Chem.* **1975**, *40*, 2138.

34. a) Porter, N. A.; Wolf, R. A.; Yarbro, E. M.; Weenen, H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 1058.

b) Porter, N. A.; Logan, J.; Kontoyiannidou, V. *J. Org. Chem.* **1979**, 44, 3177.

c) Terao, J.; Matsushita, S. *Agric. Biol. Chem.* **1981**, *45*, 587.

35. Corey, E.J., and Park, H. *J. Am Chem. Soc.* 104: 1750-1752 (1982).

36. *NCI Press Office* 1997, Recent trends in prostate cancer incidence and mortality. National Cancer Institute.

37. Petrylak 1999 *Urology* **54**: 30 -35

38. Cleeve 1999 *Prostate News, Prostate Cancer Society of Austalia,* **3**

39. Greenberg *et al* 1995 *PNAS* **92**: 3439-3443

40. Chen *et al* 1992 *Steroids*, **57**: 269-275

41. Zhao *et al* 1997 J *Biol Chem*, **272**: 22751-22757

42. Bartsch *et al* 1999 *Carcinogenesis* **20**: 2209-2218

43. Yang *et al* 1999 *Clin. Biochem*, **32**: 405-409

44. Norrish *et al* 1999 *Br J Cancer* **81**: 1238-1242

45. Hii *et al* 1995 *Carcinogenesis* **16**: 1505-1511

46. Couper *et al* 1998 *Human Immunology* **59**: 493-499

47. Hii *et al* 1995 J *Biol Chem* **270**: 4201-4204

48. Hardy *et al* 1995 *Biochem J*, **311**: 689-697

49. Huang *et al* 1997 *Biochem J*, **325**: 553-557

50. Hii *et al* 1999 in Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases (Honn, K.V., Nigam, S., Marnett, L. J. and Dennis, E. eds), pp 365-370, Plenum Press, NY.

51. Ferrante *et al* 1999 in The Neutrophils: New Outlook for the Old Cells (Gabrilovich, D. ed) **4**: 79-150, Imperial College Press.

52. Anderson *et al* 1998 *Prostate*, **37**: 161-173

53. Ghosh and Myers 1998 *PNAS*, **95**: 13182-13187

54. Palayeor *et al* 1999 *Oncogene*, **18**: 7389-7394

55. Ford-Hutchinson 1991 *Trends Pharmacol Sci*, **12**: 68-70

56. Avis *et al* 1996 *J. Clin Invest.*, **97**: 806-813

57. Australian Institute of Health and Welfare (AIHW). Breast and Cervical Cancer Screening in Australia 1996-97. 1998. Canberra, Australian Institute of Health and Welfare.

58. Johnson-Thompson, M.C. and Guthrie, J. (2000) Cancer, **88**, 1224-1229.

59 . Bartsch, H., Nair, J. and Owen, R.W. (1999) Carcinogenesis, **20**, 2209-2218.

60. Rose, D.P. and Connolly, J.M. (1997) Breast Cancer Res Treat, **46**, 225-237.

61. Steele, V.E., Holmes, C.A., Hawk, E.T., Kopelovich, L., Lubet, R.A., Crowell, J.A., Sigman, C.C. and Kelloff, G.J. (1999) Cancer Epidemiol Biomarkers Prev, **8**, 467-483.

62. Przylipiak, A., Hafner, J., Przylipiak, J., Kohn, F.M., Runnebaum, B. and Rabe, T. (1998) Gynecol Obstet Invest, **46**, 61-64.

63. Connolly, J.M. and Rose, D.P. (1998) Cancer Lett, **132**, 107-112.

64. Natarajan, R., Esworthy, R., Bai, W., Gu, J.L., Wilczynski, S. and Nadler, J. (1997) J Clin Endocrinol Metab, **82**, 1790-1798.

65. Editorial, *J. Clin. Invest.* 99: 1147-1148 (1997).

66. Wenzel, S.E. *Am. J. Med.* 104: 287-300 (1998).

67. Pitt MJ., Easton CJ. Ferrante A., Poulos, A., Rathjen DA. *Chem. Phys. Lipids* 92: 63-69 (1998).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An anti-cancer pharmaceutical composition comprising, as an anti-cancer agent, one or more compounds having the formula NO_2 – A – B, wherein A is a saturated or unsaturated hydrocarbon chain of 14 to 26 carbon atoms and B is $(CH_2)_n$ $(COOH)_m$ in which n is an integer from 0 to 2 and m is an integer from 0 to 2, and a pharmaceutically acceptable carrier or diluent.

2. A pharmaceutical composition according to claim 1, in which the hydrocarbon chain of the compound(s) includes one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

3. A pharmaceutical composition according to claim 1, in which the hydrocarbon chain of the compound(s) is either saturated or unsaturated and has 18 to 22 carbon atoms.

4. A pharmaceutical composition according to claim 1, in which the hydrocarbon chain of the compound(s) has from 3 to 6 double bonds.

5. A pharmaceutical composition according to claim 1, in which the hydrocarbon chain of the compound(s) is unsaturated and has eighteen carbon atoms and three double bonds separated by methylene groups, with the first double bond relative to the omega carbon atom being between the 3rd and 4th or 6th and 7th carbon atoms.

6. An anti-cancer pharmaceutical composition comprising, as an anti-cancer agent, the compound (all-Z)-4-nitrotricosa-8,11,14,17-tetraenoic acid or 3-[(all-Z)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic acid.

7. A pharmaceutical composition according to any one of claims 1 to 6, in which the cancer is prostate cancer.

8. A pharmaceutical composition according to any one of claims 1 to 6, in which the cancer is breast cancer.

9. A method of treating cancer in a subject, said method comprising administering to the subject a therapeutic amount of a compound having the formula NO_2-A-B, wherein A is a saturated or unsaturated hydrocarbon chain of 14 to 26 carbon atoms and B is $(CH_2)_n$ $(COOH)_m$ in which n is an integer from 0 to 2 and m is an integer from 0 to 2.

10. A method according to claim 9, in which the hydrocarbon chain of the compound includes one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

11. A method according to claim 9, in which the hydrocarbon chain of the compound is either saturated or unsaturated and has 18 to 22 carbon atoms.

12. A method according to claim 9, in which the hydrocarbon chain of the compound has from 3 to 6 double bonds.

13. A method according to claim 9, in which the hydrocarbon chain of the compound is unsaturated and has eighteen carbon atoms and three double bonds separated by methylene groups, with the first double bond relative to the omega carbon atom being between the 3rd and 4th or 6th and 7th carbon atoms.

14. A method according to claim 9, wherein the compound is (all-Z) -4-nitrotricosa-8,11,14,17-tetraenoic acid or 3-[(all-Z)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic acid.

15. A method according to any one of claims 9 to 14, in which the cancer is prostate cancer.

16. A method according to any one of claims 9 to 14, in which the cancer is breast cancer.

17. Use of a compound having the formula NO_2-A-B, wherein A is a saturated or unsaturated hydrocarbon chain of 14 to 26 carbon atoms and B is $(CH_2)_n$ $(COOH)_m$ in which n is an integer from 0 to 2 and m is an integer from 0 to 2, for the preparation of a pharmaceutical composition for the treatment of cancer.

18. Use according to claim 17, in which the hydrocarbon chain of the compound includes one or more than one substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

19. Use according to claim 17, in which the hydrocarbon chain of the compound is either saturated or unsaturated and has 18 to 22 carbon atoms.

20. Use according to claim 17, in which the hydrocarbon chain of the compound has from 3 to 6 double bonds.

21. Use according to claim 17, in which the hydrocarbon chain of the compound is unsaturated and has eighteen carbon atoms and three double bonds separated by methylene groups, with the first double bond relative to the omega carbon atom being between the 3rd and 4th or 6th and 7th carbon atoms.

22. Use according to claim 17, wherein the compound is (all-Z) -4-nitrotricosa-8,11,14,17-tetraenoic acid or 3-[(all-Z)-nonadeca-4,7,10,13-tetraenyl]-3-nitropentane-1,5-dicarboxylic acid.

23. Use according to any one of claims 17 to 22, wherein the cancer is prostate cancer.

24. Use according to any one of claims 17 to 22, wherein the cancer is breast cancer.

25. An anti-cancer pharmaceutical composition comprising, as an anti-cancer agent, one or more compounds selected from polyunsaturated fatty acids having a 16 to 26 carbon atom chain and 3 to 6 double bonds, and wherein the polyunsaturated fatty acid is covalently coupled at the carboxylic acid group to an amino acid selected from glycine and aspartic acid, and a pharmaceutically acceptable carrier or diluent.

26. A method of treating cancer in a subject, said method comprising administering to the subject a therapeutic amount of a compound selected from polyunsaturated fatty acids having a 16 to 26 carbon atom chain and 3 to 6 double bonds, and wherein the polyunsaturated fatty acid is covalently coupled at the carboxylic acid group to an amino acid selected from glycine and aspartic acid.

27. Use of a compound selected from polyunsaturated fatty acids having a 16 to 26 carbon atom chain and 3 to 6 double bonds, and wherein the polyunsaturated fatty acid is covalently coupled at the carboxylic acid group to an amino acid selected from glycine and aspartic acid, for the preparation of a pharmaceutical composition for the treatment of cancer.

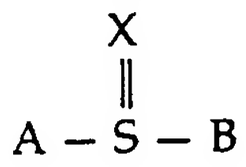
28. An anti-cancer pharmaceutical composition comprising, as an anti-cancer agent, one or more compounds selected from unsaturated fatty acids having an 18 to 25 carbon atom chain and 1 to 6 double bonds and wherein the fatty acid has one or two substitutions selected from the group consisting of β-oxa, γ-oxa, β-thia and γ-thia, and a pharmaceutically acceptable carrier or diluent.

29. A method of treating cancer in a subject, said method comprising administering to the subject a therapeutic amount of a compound selected from unsaturated fatty acids having an 18 to 25 carbon atom chain and 1 to 6 double bonds

and wherein the fatty acid has one or two substitutions selected from the group consisting of β-oxa, γ-oxa, β-thia and γ-thia.

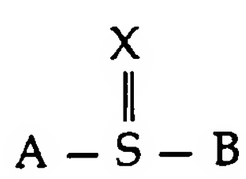
30. Use of a compound selected from unsaturated fatty acids having an 18 to 25 carbon atom chain and 1 to 6 double bonds and wherein the fatty acid has one or two substitutions selected from the group consisting of β-oxa, γ-oxa, β-thia and γ-thia, for the preparation of a pharmaceutical composition for the treatment of cancer.

31. An anti-cancer pharmaceutical composition comprising, as an anti-cancer agent, one or more compounds selected from compounds having the formula



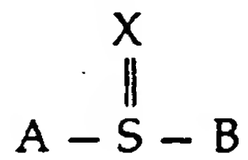
wherein A is a saturated or unsaturated hydrocarbon chain of 9 to 26 carbon atoms, X is oxygen or is absent and B is $(CH_2)_j(COOH)_k$ in which j is an integer from 1 to 3 and k is 0 or 1, and derivatives thereof in which the hydrocarbon chain includes one or more substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy, and a pharmaceutically acceptable carrier or diluent.

32. A method of treating cancer in a subject said method comprising administering to the subject a therapeutic amount of a compound having the formula



wherein A is a saturated or unsaturated hydrocarbon chain of 9 to 26 carbon atoms, X is oxygen or is absent and B is $(CH_2)_j(COOH)_k$ in which j is an integer from 1 to 3 and k is 0 or 1, or a derivative thereof in which the hydrocarbon chain includes one or more substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy.

33. Use of a compound selected from compounds having the formula



wherein A is a saturated or unsaturated hydrocarbon chain of 9 to 26 carbon atoms, X is oxygen or is absent and B is $(CH_2)_j(COOH)_k$ in which j is an integer from 1 to 3 and k is 0 or 1, or a derivative thereof in which the hydrocarbon chain includes one or more substitution selected from the group consisting of hydroxy, hydroperoxy, epoxy and peroxy, for the preparation of a pharmaceutical composition for the treatment of cancer.

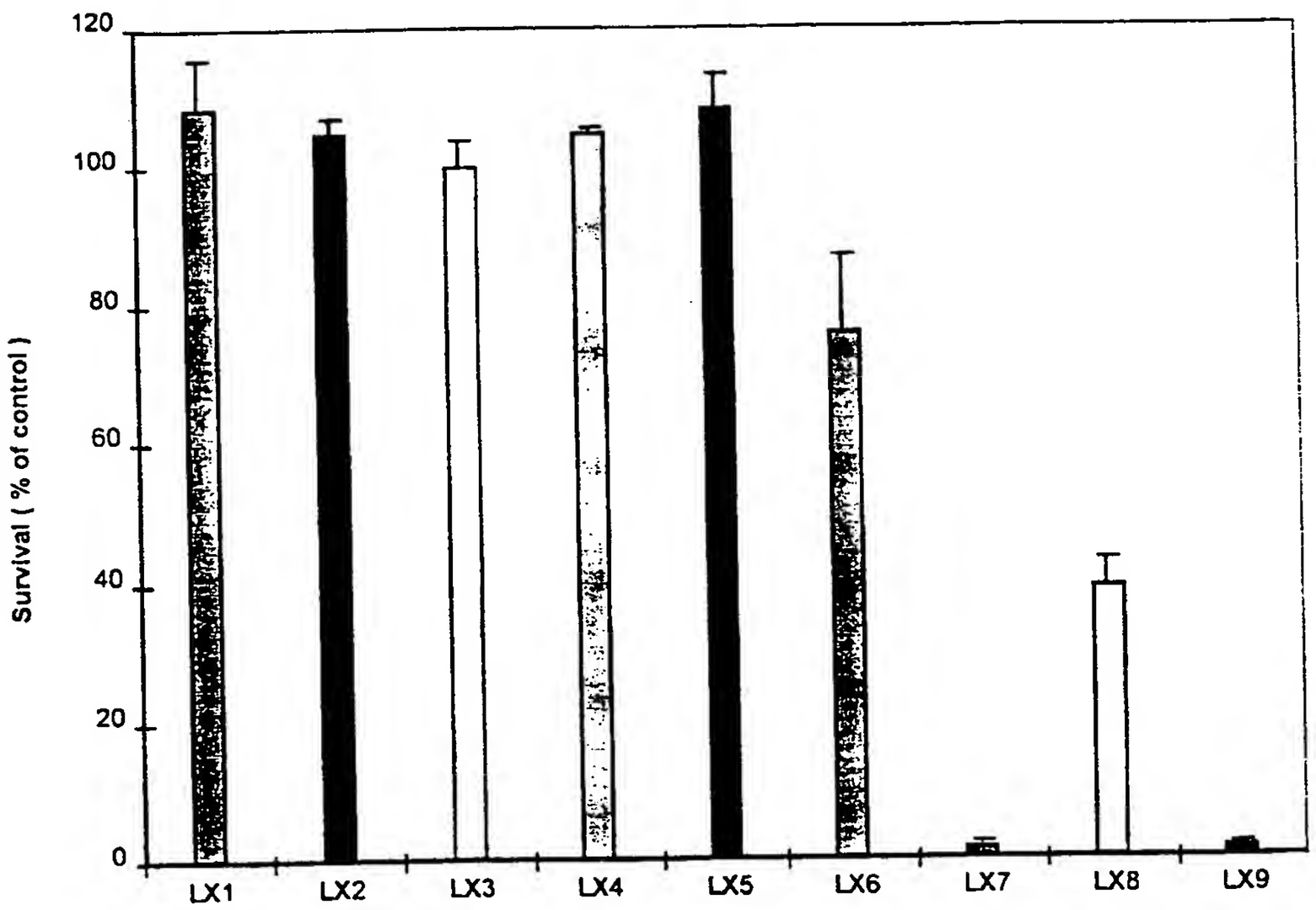
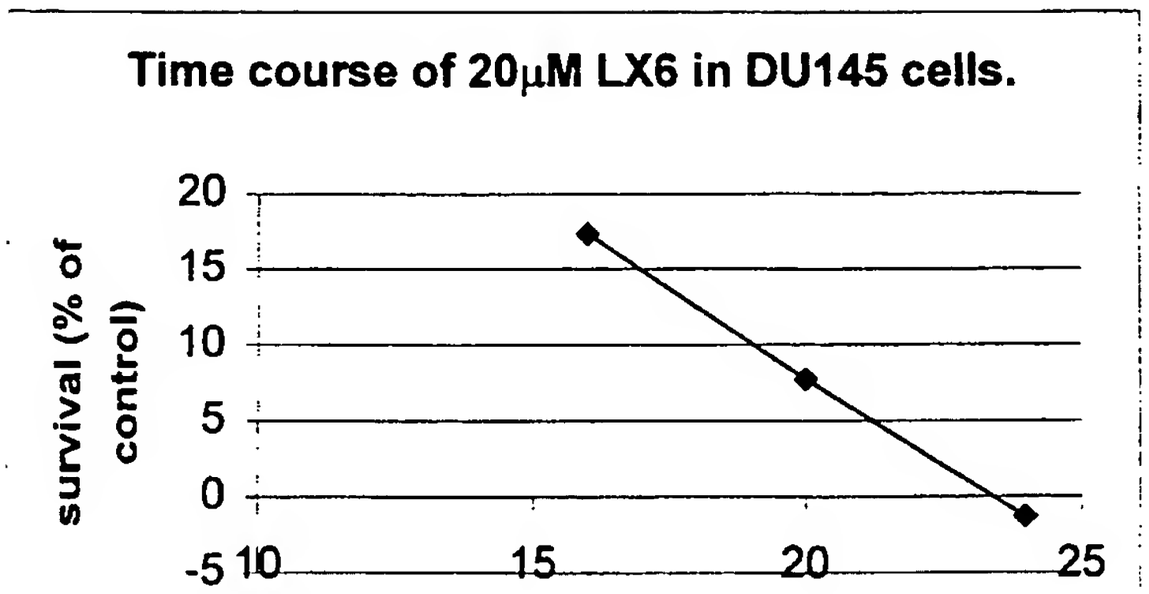

120
100
80
60
40
20
0
Survival (% of control)
LX1
LX2
LX3
LX4
LX5
LX6
LX7
LX8
LX9
Compound

FIGURE 1



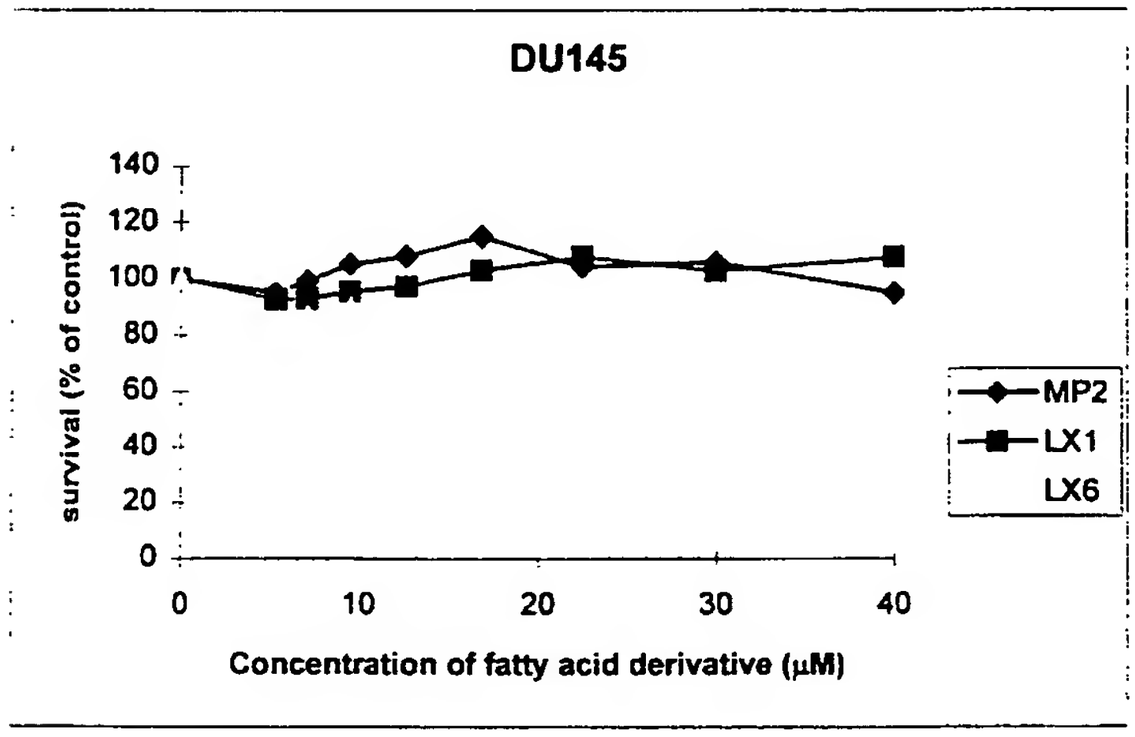


FIGURE 2

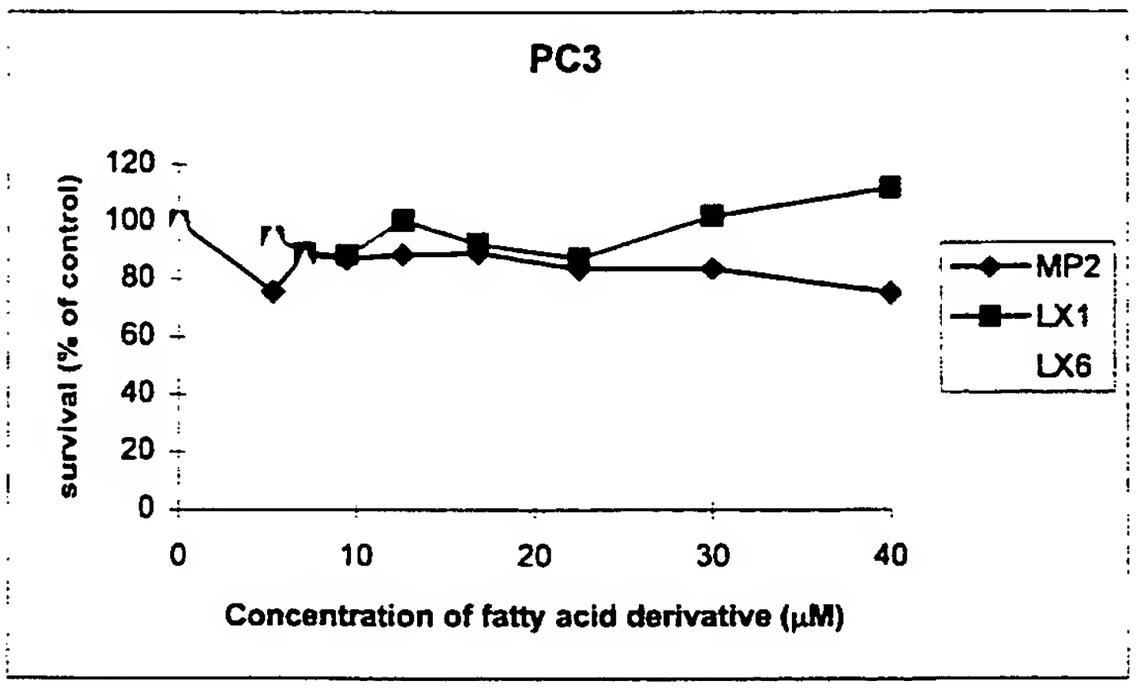


FIGURE 3

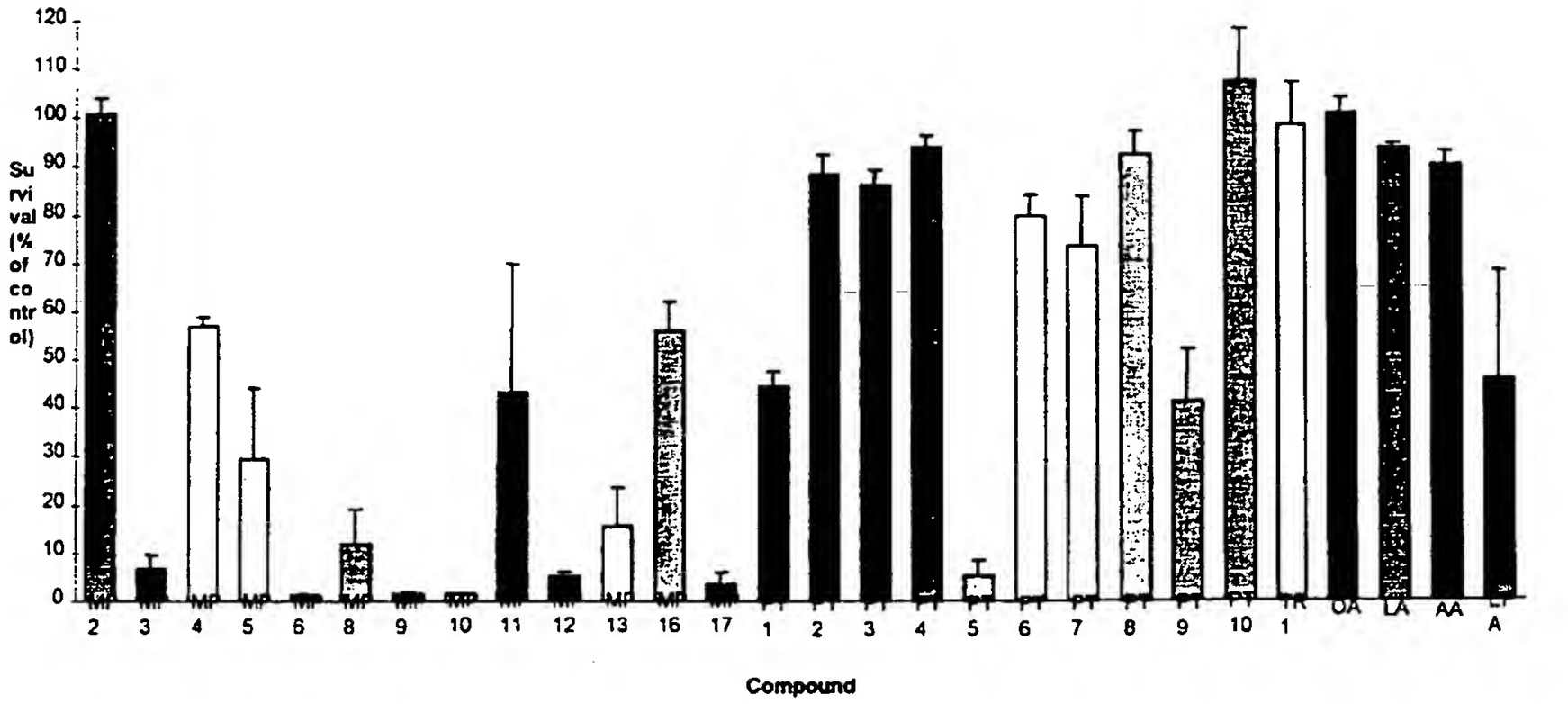


FIGURE 4

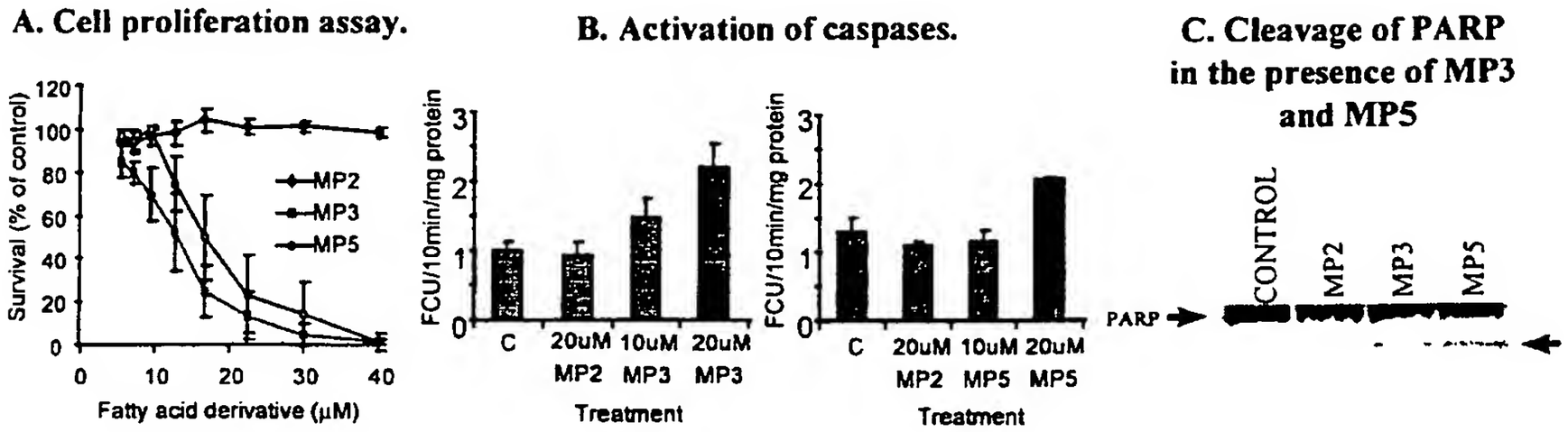
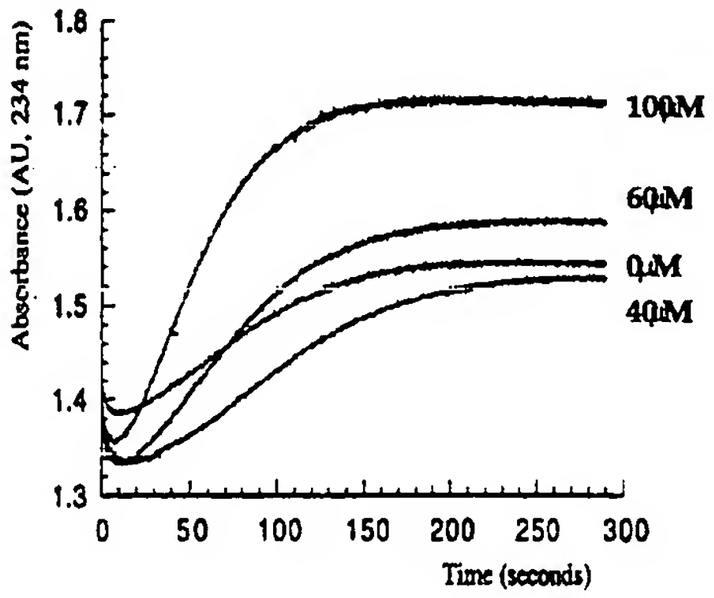
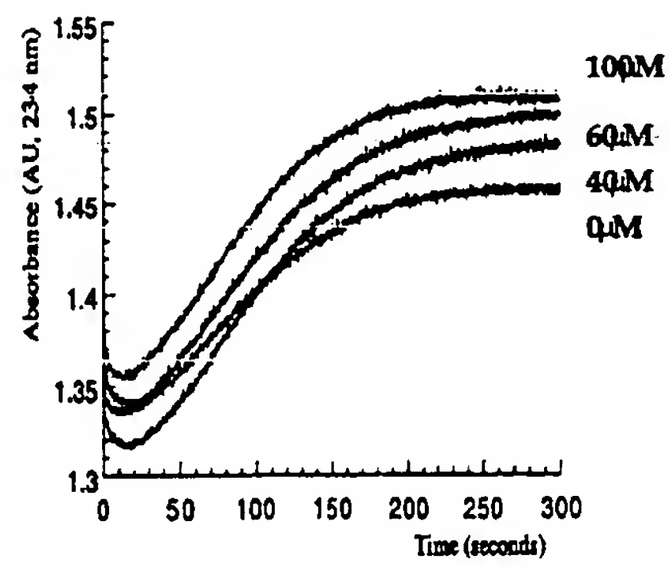


FIGURE 5

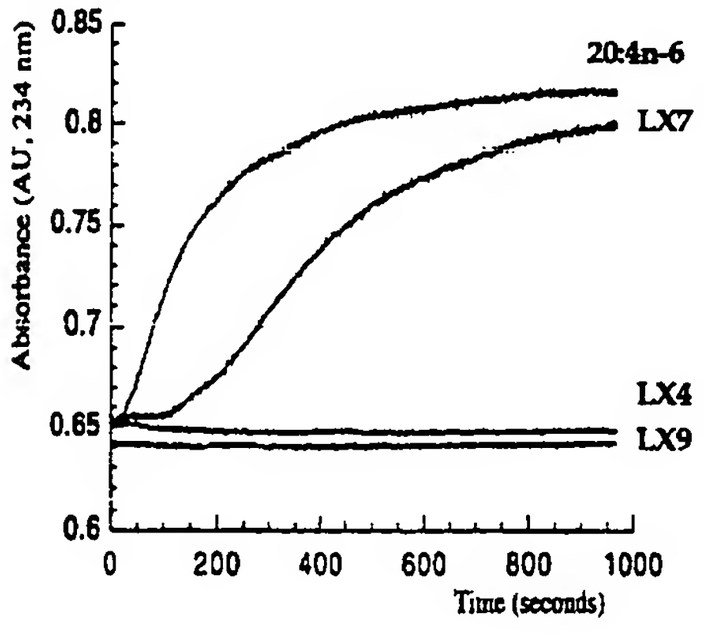
A. Activation of 5-LOX by



B. Activation of 5-LOX by



C. LX7 is a substrate for 12-LOX



D. LX9 inhibits 12-LOX

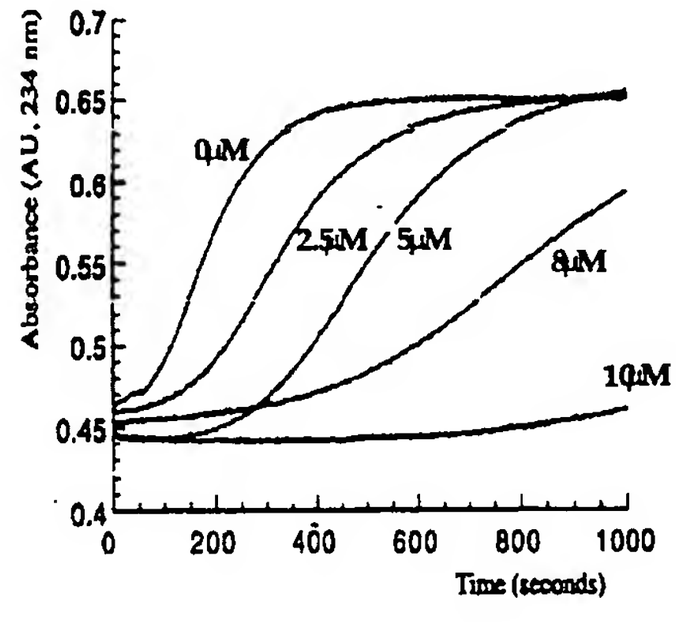


FIGURE 6

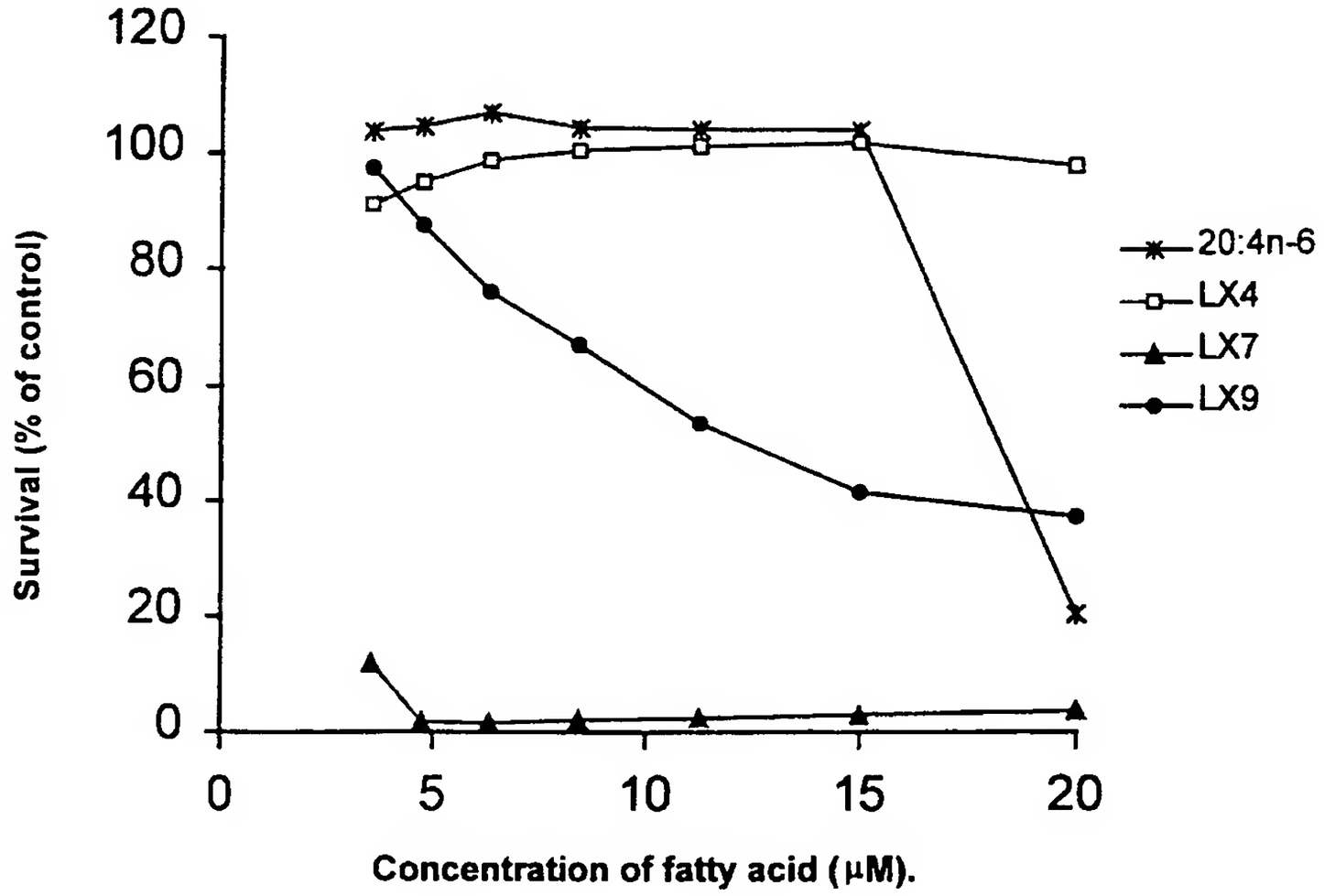

120
100
80
60
40
20
0
Survival (% of control)
0
5
10
15
20
Concentration of fatty acid (μM).
20:4n-6
LX4
LX7
LX9

FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01137

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/20, 31/202, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7: AS ABOVE.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Structure search intersected with key word (cancer) search on STN.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/03663 A (BERGE, Rolf) 25 October 1995 See the abstract, the claims and Table 4 (tetradecylthioacetic acid).	31 to 33
X	DE 2208533 A (KOLMAR Laboratories) 27 September 1973 See examples 3 (n-nonylthioacetic acid) and 4 (3,7-dimethyloctylthioacetic acid).	31 to 33

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2000	14 DEC 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **GAVIN THOMPSON** Telephone No : (02) 6283 2240

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/01137

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member				
WO 97/03663	AU 42726/96	CA 2226871	EP 840604	JP 11514339	NO 952796
	US 6046237				
DE 2208533					

END OF ANNEX